

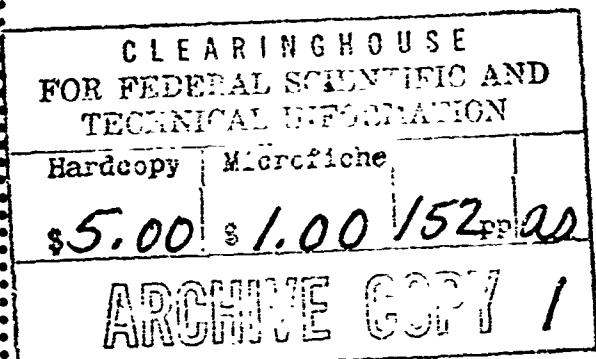
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MISCELLANEOUS PUBLICATION 3

IMMUNOFLUORESCENCE,
AN ANNOTATED BIBLIOGRAPHY
IV. STUDIES OF ANIMAL PHYSIOLOGY

Warren R. Sanborn



DECEMBER 1965

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Warren R. Sanborn

December 1965

Pathology Division
DIRECTORATE OF MEDICAL RESEARCH

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FOREWORD

The use of immunofluorescence, or fluorescent antibodies, was initiated by Dr. Albert H. Coons and his co-workers in 1942. Dr. Coons has modestly stated that making antibodies fluorescent was "simply another variation of their use as reagents for the identification of specific antigen. . . ." However, this "variation" has proved to be one of immense significance to modern immunology. Its importance lies in the wedding of the two broad areas of investigation, morphology and immunology, thus allowing the detection of immunologic reactions at the cellular level.

The expanding volume of literature covering uses of immunofluorescence bears witness to the value of the technique. Through 1954 only about 40 articles had been published. In the next two years 58 articles were published. In 1957 and 1958 there were 83 and 96, respectively. By 1961 this figure had risen to more than 260 in that one year alone. Apparently more than 400 articles per year can be expected for 1964 and 1965.

It would be virtually impossible to cite every article that refers to the use of immunofluorescence, but an attempt has been made in this six-volume annotated bibliography. Fifteen languages are represented, and more than 150 journals have been searched. Six abstracting journals have been included in the search. Translations were provided by several co-workers, government translating services, and the compiler. The earliest entry is dated 1905; significant publications through 1962 are included. Subsequent entries are being compiled and will be incorporated into revisions of this bibliography. The additions will, no doubt, increase considerably the bulk of these volumes.

The bibliography is intended to aid investigators in following the expanding mass of literature on the technique and to improve their skill in its use. The entire publication, Miscellaneous Publication 3, carries the title: "Immunofluorescence, an Annotated Bibliography." The subtitles for the six volumes are: Volume I, "Bacterial Studies"; Volume II, "Viral Studies"; Volume III, "Studies of Fungi, Metazoa, Protozoa, and Rickettsiae"; Volume IV, "Studies of Animal Physiology"; Volume V, "Diagnostic Applications and Review Articles"; and Volume VI, "Technical Procedures." Each of the volumes is subdivided into subject categories that should, hopefully, aid the reader in finding pertinent information in his field of interest without his spending undue time in scanning superfluous citations. Articles within subject categories are arranged alphabetically by senior author.

Accession numbers in each volume were assigned to articles by tens to allow room for expansion in subsequent editions. Accession numbers within each volume are consecutive throughout that volume, so the volume number must accompany the accession number to identify an entry unmistakably. Entries applicable to more than one subject category appear more than once, and these will have an accession number for each placement in the volumes.

A complete author index is included in each volume; the author's name is listed with the accession numbers of the entries with which he is associated. The asterisk designates those for which he is senior author.

The second parts of Volumes V and VI contain only references to articles printed in the other four volumes. As in the other volumes, the references are placed in subject categories, and are arranged alphabetically by senior author within categories. The authors, the year of publication, and the volume and accession number are shown to indicate where the complete entry can be found.

For brevity, certain abbreviations in common usage in this field have been used rather than the more ponderous longer form. For unmistakable identification, they are listed below.

DANS	a. 1-dimethylaminonaphthalene-5-sulfonic acid b. 5-dimethylamino-1-naphthalene sulfonic acid or its sulfonyl chloride form.
FIC	fluorescein isocyanate
FITC	fluorescein isothiocyanate
FTA	fluorescent treponelial antibody
FTA-200	a modification of the above based on serum dilution.
PAP	primary atypical pneumonia
PAS	para-aminosalicylic acid
PBS	phosphate-buffered saline
RB 200	a. lissamine rhodamine RR 200 b. lissamine rhodamine B 200 c. lissamine rhodamine B d. sulphorhodamine B e. acid rhodamine B
TPFA	<u>Treponema pallidum</u> fluorescent antibody
TPI	<u>Treponema pallidum</u> immobilization

Generally, the citations follow the format prescribed by the second edition of "Style Manual for Biological Journals," American Institute of Biological Sciences, 2000 P Street, N.W., Washington, D.C., 20036. Abbreviations follow "American Standard for Periodical Title Abbreviations," Z39.5-1963, American Standards Association Incorporated, New York.

The compiler started collecting this information in 1959 while he was stationed at the U.S. Army Biological Laboratories, Fort Detrick, Frederick, Maryland. Since his transfer to the Naval Medical Research Institute, Bethesda, Maryland, in 1963, he has continued this work with the encouragement and support of both installations.

The information in these volumes was originally recorded on coded Keysort cards. With the compilation of this publication, the citations and annotations have been transcribed on punched tape for conversion to automatic data processing and for use in updating later editions. Each entry is coded for recall by authors, date, title, and source publication to allow compilation of more selective listings.

Readers are invited to report errors or suggest added entries to the compiler or to Editorial Branch, Technical Information Division, U.S. Army Biological Laboratories, Frederick, Maryland, 21701, for improvement of the subsequent editions.

ACKNOWLEDGMENTS

This compilation would have been much more difficult if not impossible without the guidance, help, and encouragement of:

Dr. Harold W. Batchelor, who introduced the compiler to card-sorting systems;

The staff of Naval Medical Research Institute, who provided both support and personnel for assistance in this work;

My colleagues at the U.S. Army Biological Laboratories and the Walter Reed Army Unit at Fort Detrick and at the Naval Medical Research Institute in Bethesda, who volunteered their technical competence and supplied moral support.

Obviously, outstanding cooperation and assistance of librarians was required for this work. The staff of the Technical Library, Fort Detrick, under the direction of Mr. Charles N. Bebee, was continually patient, understanding, and essential for the entire period of compilation.

Another essential in the chain to final publication: Mrs. Madeline D. Warnock and her staff in the Editorial Branch at Fort Detrick have served as editors, of course, but also as confessors, encouragers, consciences, and the required driving force, all of which have brought this to its publication. The compiler is most grateful to Mrs. Warnock and her people.

ABSTRACT

This volume is one of a series of six annotated bibliographies on various aspects of immunofluorescence and its use. Citations cover the period 1905 through 1962. Volume IV contains 432 annotated literature citations, arranged according to major subject areas, and a complete author index.

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I. COLLAGEN DISEASE

10

Alexander, W.R.M.; Bremner, J.M.; Duthie, J.J.R. 1960. Incidence of the antinuclear factor in human sera. Ann. Rheum. Dis. 19:338-350.

A sensitive method employing FA is described for the detection of antinuclear factor, ANF, in human serum in 1,116 cases. ANF was present in all disseminated lupus erythematosus patients as well as in some cases of polyarteritis nodosa, dermatomyositis, and scleroderma. It was present in 65 per cent of rheumatoid arthritis patients and in varying percentages of patients with other arthritic conditions, a variety of other diseases, and in 4 per cent of apparently normal individuals. The incidence of ANF in rheumatoid arthritics was highest in patients with active disease. The possible significance of these results is discussed.

20

Alexander, W.R.M.; Potter, J.L. 1961. Antinuclear factor. Lancet 1:830-831.

Using the Coons technique for demonstrating antinuclear factor and assuming the reaction to be antigen-antibody in nature may lead to false conclusions. Labeled plant and animal enzymes in some instances also show an affinity for nuclei.

30

Aubuchon, M. 1959. Labeling antibodies. Hospital Progr. 40:122-123.

The use of the fluorescent antibody technique and its development through the years is reported. Recent work to prove that the stimulation of antibody production in rheumatic fever cases causes possible heart damage is included.

40

Bardawil, W.A.; Pachas, W.N.; Sbarra, A.J.; Turrubiarte, V. 1962. Antinucleolar globulins in collagen disease. Federation Proc. 21:12.

In a search for antinuclear globulins in patients afflicted with collagen diseases, we have found, in addition, in six different instances, globulins with reactive affinity for nucleoli. This reaction, as demonstrated by the direct and indirect fluorescent antibody technique, is organ- and species-nonspecific. This is indicated by the fact that they bind nucleoli from human skin, placenta, carcinoma of the stomach, colon, and a Wilms tumor. The reaction is abolished by prior digestion of tissues with RNAase or by absorption of the serum with yeast RNA. DNAase treatment of the tissues or absorption of the serum with animal DNA or histone does not alter the reaction. These findings confirm and extend the report of Rodnan and Fennell in that patients suffering not only from scleroderma, but also from other diseases, exhibited antinucleolar globulins. Our cases included one with disseminated lupus erythematosus, a case of pulmonary fibrosis, and one of rheumatoid arthritis in addition to three cases of scleroderma.

10

50

Bardawil, W.A.; Toy, B.L.; Galins, N. 1958. Hypersensitivity to histone, induced experimentally in rabbits. Lancet 1:888-889.

The histochemical behavior of sera from human patients with certain so-called collagen diseases has been simulated by samples from an animal sensitized against bovine histone. Evidence is presented that the observed effect depends on immunological reaction, and the relation to human disease is discussed.

60

Bardawil, W.A.; Toy, B.L.; Galins, N.; Bayles, T.B. 1958. Disseminated lupus erythematosus, scleroderma, and dermatomyositis as manifestations of sensitization to DNA protein: I. An immunohistochemical approach. Amer. J. Pathol. 34:607-629.

A peculiar binding trait of gamma globulin has been observed in patients with disseminated lupus erythematosus, scleroderma, and dermatomyositis. By the use of fluorescent antibody techniques, this serum factor has been shown to possess a reactive affinity with intranuclear material, probably deoxyribonucleoprotein, within autologous, homologous, and heterologous tissues. The findings are correlated and prompt the suspicion that the above diseases, and perhaps related conditions such as rheumatoid arthritis, may be manifestations of a common disease process initiated by sensitization against either intrinsic or extrinsic nucleoprotein. Nuclear binding appears to require the participation of DNA but not RNA, and complement probably is not involved in the in vitro phenomenon. The pathogenesis of so-called diseases of collagen, as represented by those investigated, is discussed briefly.

70

Barnes, R.; Carmichael, D.; Johnson, G.D. 1962. Comparison between the latex nucleoprotein test and the fluorescent method for the demonstration of antinuclear factor. Ann. Rheum. Dis. 21:287-291.

A comparative study of the results obtained by the fluorescent antibody method for the detection of serum antinuclear factor and a commercially available latex nucleoprotein reagent has been carried out on a series of 525 sera. The latex nucleoprotein test was positive in only 8 of 24 cases of clinically defined systemic lupus erythematosus, in all of which the fluorescent test was positive. The latex nucleoprotein test was positive in two individuals in whom the fluorescence test was negative - in a case of discoid lupus and in a relative of a patient with an undefined collagen disease. To account for the relative hyposensitivity of the latex nucleoprotein reaction, we have considered the possibility of inhibition by free DNA, which we have shown to be present in the latex reagent. When coated particles resuspended in DNA-free buffer were substituted in the test, however, no significant increase in sensitivity was found. It does not therefore appear that this test is a satisfactory substitute for the immuno-fluorescent procedure in the detection of serum antinuclear factor.

80

Barton, E.M. 1959. Abnormal serum proteins as aids in diagnosis of rheumatoid arthritis and systemic lupus erythematosus. *Med. Clin. N. Amer.* 43:607-613.

Evidence exists for relatively specific serum factors in both rheumatoid arthritis and systemic lupus erythematosus. They are globulin components of quite different molecular size; the rheumatoid factor is a macroglobulin and the lupus factor is much smaller. They have been characterized and may be identified by numerous biologic, immunologic, and physicochemical methods among which there is general agreement. Cross-reactions between the two diseases are apparently due to the presence of both factors in such sera, although one usually predominates. Until the origin and significance of these abnormal proteins are firmly established it would be unwise to abandon well-established clinical criteria for diagnosis merely because of confusing serology. Each test must be used and evaluated in the clinical spectrum presented by patients, whether these be individuals or related groups.

90

Baugh, C.W.; Kirol, P.M.; Sachs, M.V. 1960. Demonstration and titration of anti-nuclear antibodies in systemic lupus erythematosus. *Can. Med. Ass. J.* 83:571-582.

Fluorescent antibody was employed to study a factor with affinity for calf thymic nuclei in the serum of patients with systemic lupus erythematosus, SLE, and other diseases. An attempt was made to quantify the amount of serum factor by titer determinations. High titers were encountered in the serum of patients with multiple clinical manifestations of SLE and a history of a positive LE cell test. The sensitivity of the method was compared with the results obtained with LE cell preparation tests performed on the same sample of serum. In 39 cases of patients who at some time had a positive LE cell preparation, the LF cell preparation was positive in 20 at the time of study, whereas 35 cases showed significant titers of antinuclear antibody. Positive results were also seen in three cases of possible SLE and in five cases with diseases seemingly related to SLE. One apparently false positive result was obtained. High serum titers were found in 13 cases showing active disease, but also in 12 cases with slight activity or in remission. In only two cases was there a low titer associated with active disease.

100

Baum, J.; Ziff, M. 1962. 7S and macroglobulin antimuclear fluorescence factors in systemic lupus erythematosus and rheumatoid arthritis. *Arth. Rheum.* 5:636.

Sera from 36 patients were fractionated on DEAE cellulose: 9 SLE patients with rheumatoid factor, 8 SLE patients without rheumatoid factor, and 19 rheumatoid arthritis patients with ANF. ANF distributions in the fractions were studied by indirect FA. ANF was found in peak I, 0.01 M phosphate at pH 7.0, and in peak IV, 0.3 M phosphate at pH 5.0, corresponding to 7S and 19S gamma globulins. Mercaptoethanol abolished activity in peak IV but not in I. Of the 17 SLE patients, ANF was in peak I in twelve and in peaks I and IV in five. In the rheumatoid arthritis patients ANF was in peak I alone in one, I and IV in six, and IV alone in twelve. Chromatography of mixtures of SLE sera containing peak I ANF plus high-titered rheumatoid factor sera containing no ANF was performed to rule out the possibility of interaction between ANF and rheumatoid

factor. There was no interaction. ANF occurs in 7S and 19S gamma globulins. In SLE patients the ANF is mainly in the 7S fractions; in rheumatoid arthritis patients with ANF the activity is mainly in the 19S fraction.

110

Beck, J.S. 1961. Variations in the morphological patterns of autoimmune nuclear fluorescence. *Lancet* 1:1203-1204.

Three patterns of autoimmune nuclear fluorescence have been identified in the serum of patients with Sjogren syndrome, systemic lupus erythematosus, and other possible autoimmune diseases. Homogeneous nuclear staining is produced by an antinuclear factor specific for deoxyribonucleoprotein. The antigen responsible for speckled nuclear staining has not yet been identified. Nucleolar staining, which was produced by one serum specimen, possibly represents an antinuclear factor that reacts with the structural ribonucleic acid of the nucleolus.

120

Beutner, E.H.; Nagy, C.F. 1962. The role of desiccation in immunofluorescent staining with nuclear antibodies. *J. Histochem. Cytochem.* 10:686.

Immunofluorescent staining with nuclear antibodies poses significant technical problems. Diffusion of nuclear antigen occurs frequently, leading to staining patterns that might result in misleading interpretations, i.e., staining that is immunologically specific but histologically artifactual. This phenomenon is attributable to the solubility of nucleohistones in hypertonic saline. Treatment of tissue sections with 1 M or 2 M NaCl gives rise to diffuse staining with nuclear antibodies. If, in the course of processing, partial desiccation of the saline overlaying the sections occurs, then the resulting hypertonic solution dissolves nuclear antigen; this gives rise to erratic immunofluorescent staining patterns. Thus, rigorous care must be exercised to avoid drying in staining with nuclear antibodies. Upon complete drying of the saline overlaying tissue sections, characteristic changes occur. Monkey liver sections treated in this way become completely negative for nuclear staining, but a portion of the nuclear antigen in monkey brain sections remains intact.

130

Braunsteiner, H.; Rigler, R.; Pakesch, F. 1962. Immunofluorescence investigation of bone marrow cells in primary chronic polyarteritis. *Klin. Wochensch.* 40:22:1139-1141. In German.

In bone-marrow smears of patients with primary chronic polyarteritis the rheumatoid factor can be demonstrated with fluorescent aggregated gamma globulin in matured plasma cells, as well as, singled out, in reticular cells, for example in plasma cellular rudiments. Ten to 30 per cent of plasma cells show fluorescence. In latex-negative cases fluorescent plasma cells can be found to a much lesser degree, 5 to 10 per cent. On the basis of these studies a special position for latex-negative cases in primary chronic polyarteritis cannot be derived.

140

Calabresi, P.; Edwards, E.A.; Schilling, R.F. 1959. Fluorescent antiglobulin studies in leukopenic and related disorders. *J. Clin. Invest.* 38:2091-2100.

A method for studying leukocyte immunology by the fluorescent antiglobulin technique is described. The results with sera from various disease states are reported. The data presented are consistent with the concept that human antileukocyte globulins may be directed against the nucleus or against the cytoplasm. Antinuclear globulins were detected in the sera of all patients in this study who had systemic lupus erythematosus and the Felty syndrome, and in two cases of apparently uncomplicated rheumatoid arthritis. These findings suggest that the Felty syndrome may be a connecting link of a disease spectrum involving simple rheumatoid arthritis and SLE. Evidence that a circulating factor present in patients with the Felty syndrome may be involved in the pathogenesis of the leukopenia was obtained in two instances by plasma transfusions.

150

Calabresi, P.; Finch, S.C. 1962. The value of the fluorescent antiglobulin technic in the differential diagnosis of aleukemic leukemia. *Proc. 8th Congr. Int. Soc. Blood Transfus.*, Tokyo. p. 364.

FA was applied to leukopenic patients to differentiate aleukemic leukemias from other conditions. Antinuclear factors were demonstrated in patients with a variety of disorders. The presence of antinuclear globulin in leukopenic patients may exclude diagnoses of aleukemic leukemia and lymphosarcoma.

160

Calabresi, P.; Greenberg, M. 1960. Circulating antinuclear globulins in patients with chronic liver disease. *J. Clin. Invest.* 39:976.

Sera of 32 chronic liver disease patients, 24 alcoholics with cirrhosis, and 8 with post-necrotic cirrhosis were tested for antinuclear globulins by the fluorescent antiglobulin technique. Nuclear material was from fixed homologous leukocytes in normal peripheral blood smears. Ten patients were positive. Positives were found in 50 per cent of the women and 17 per cent of the men. No clinical differences were noted between positives and negatives. The latex fixation test was positive in each positive antinuclear test. It was concluded that abnormal circulating nuclear-specific globulin may be present in some patients with chronic liver disease.

170

Calabresi, P.; Thayer, W.R.; Spiro, H.M. 1961. Demonstration of circulating anti-nuclear globulins in ulcerative colitis. *J. Clin. Invest.* 40:2126-2133.

Sera from 24 patients with ulcerative colitis were studied for the presence of anti-nuclear globulins by the fluorescent antiglobulin technique. In three-fourths of the patients a positive reaction was detected on whole nuclei of human leukocytes, but not on calf thymus nucleoprotein. Tests for rheumatoid factor and lupus erythematosus were negative. Antinuclear factors were usually present in patients with splenomegaly,

arthritis, or arthralgia and long-standing disease, but absent in 9 of 13 patients who had previously undergone colectomy for their disorders, including two who had positive tests before surgery. Although positive antinuclear tests were present in 9 patients on ACTH-corticosteroid therapy, two became negative during treatment. Positive anti-nuclear tests were encountered in systemic lupus erythematosus, rheumatoid arthritis, and related collagen disorders, as well as in chronic hepatitis cirrhosis and in some drug reactions. The presence of a circulating antinuclear globulin in ulcerative colitis is of interest.

180

Casals, S.P.; Friou, G.J.; Teague, P. 1962. Varieties of antinuclear factors detected by fluorescent antiglobulin technics. Arth. Rheum. 5:640-641.

Antinuclear factors were studied by FA, including DNA inhibition tests, using calf thymus nucleoprotein, cells from human buccal mucosa, and human peripheral blood. Specific complement-fixation tests were also done. Three identifiable staining patterns have been found that correlate with results obtained by other methods. In one, nuclear staining is predominantly marginal and a shaggy nuclear outline is produced by irregular protruding strands. This is inhibited by DNA, and these sera give positive DNA complement-fixation tests. A second pattern, not inhibited by DNA, consists of homogeneous staining with sharply demarcated nuclear borders. These sera yield positive tests with calf thymus nucleoprotein, but DNA complement-fixation tests are negative. In a third type the nuclear staining is speckled and tests with nucleoprotein and DNA are both negative. By this approach we have been able to use fluorescent antiglobulin to separate the two antinuclear antibodies most characteristic of SLE and identify a still different nuclear staining pattern in sera from other patients. An attempt is being made to identify individual factors when present simultaneously with others in a single serum and to further elucidate the nature of the factor responsible for the speckled pattern.

190

Couchman, K.; Doniach, D.; Rickett, I.M. 1961. Antinuclear factor. Lancet i:669-670.

The reaction temperature in the fluorescent antinuclear factor, ANF, test is discussed. At 37°C all tests were negative, but at 4°C no tests were negative. The obscure nature of cold-reacting ANF is discussed.

200

Craig, J.M.; Gitlin, D. 1957. The nature of the hyaline thrombi in thrombotic thrombocytopenic purpura. Amer. J. Pathol. 33:251-265.

The thrombi in two cases of thrombotic thrombocytopenic purpura were found to react specifically with fluorescein-labeled rabbit and antihuman fibrin antibodies, but were found to be negative or only occasionally and partially positive for fibrin when the tissues were fixed, embedded in paraffin, and stained by standard dye-staining procedures. These thrombi failed to react with fluorescein-labeled rabbit antihuman platelet antibodies. It is concluded that the hyaline thrombi in thrombotic thrombocytopenic purpura are composed of a saline-insoluble derivative of fibrinogen, or fibrin.

210

Crawford, H.J.; Wood, R.M.; Lessef, M.H. 1959. Detection of antibodies by fluorescent spot technique. *Lancet* 2:1173-1174.

The fluorescent spot technique for the detection of antibodies by gross observation under UV light is described. The antigens tested were thyroglobulin and nucleoprotein. Sera used were from thyroid disease patients and normal blood donor control sera for the thyroglobulin, and sera for the nucleoprotein were from systemic lupus erythematosus patients with control sera from blood donors and rheumatoid arthritis patients. All clinically positive patients yielded positive serological results, most of the undiluted controls were negative, and all were negative in dilutions of 1:10. The fluorescent-spot technique offers a rapid screening test for systemic lupus erythematosus.

220

Duheille, J.; Herbeauval, H.; Bellut, F.; Badonnel, Y. 1962. Immunofluorescent demonstration of the factors of anti-leukocyte sera. *Compt. Rend. Soc. Biol.* 156:2093-2099.

The application of direct and indirect FA to the immunology of leukocytes is described. These methods are particularly useful for lupus erythematosus.

230

Faivre, G.; Gilgenkrantz, J.M. 1962. Value of immunofluorescence in cardiology. *Actualites Cardiol. Int.* 11:15-18. In French.

The theories for immunologic phenomena in cardiac diseases are reviewed. The contributions of FA by Kaplan are discussed.

240

Fennell, R.H.; Rodnan, G.P.; Vazquez, J.J. 1962. Variability of tissue-localizing properties of serum from patients with different disease states. *Lab. Invest.* 11:24-31.

The sera from patients with systemic lupus erythematosus, scleroderma, rheumatoid arthritis, Hashimoto thyroiditis, malignant hypertension, and dermatomyositis were tested for the presence of nuclear-localizing antibodies by using the fluorescent antibody technique. Three of 21 serum specimens from patients with rheumatoid arthritis and 2 of 12 serum specimens from patients with Hashimoto thyroiditis were found by this technique to contain nuclear-localizing factors identical to the factor found with lupus serum. The serum specimens from other patients showed variable or negative results. It is suggested that the presence of nuclear-localizing factors is not a basis for pathogenetic classification of disease, and the development of the factor or factors may be a secondary phenomenon.

250

Freedman, P. 1962. Immunological studies in glomerulonephritis. Guys Hosp. Rep. 111:370-377.

Gamma globulin has been found in a high proportion of glomerular lesions in glomerulonephritis, and in renal involvement in systemic lupus erythematosus, progressive systemic sclerosis, polyarteritis nodosa, amyloidosis, and diabetes mellitus. The glomerular-localized gamma globulin may be dissociated from combination with the tissues at pH 3.2. Glomerular-bound gamma globulin obtained by elution from isolated lupus glomeruli possesses antibody-like activity in the presence of fresh normal human serum. No antibody activity could be demonstrated in gamma globulin derived from glomerulonephritic glomeruli. No evidence was found for a circulating antibody in glomerulonephritis and the connective tissue diseases except for systemic lupus erythematosus, in which a circulating antinuclear antibody was demonstrable in virtually all patients. In kidney sections, complement was localized in glomerular lesions, together with gamma globulin, in glomerulonephritis, lupus nephritis, and in one patient with diabetic nephropathy. These findings provide support for belief that an immunological reaction is proceeding in the kidney in glomerulonephritis and in renal involvement in connective tissue diseases. Significance of findings in diabetic nephropathy remains to be evaluated.

260

Friou, G.J. 1958. Laboratory and clinical evaluation of two techniques for measuring lupus globulin nucleoprotein interaction. American Rheumatism Association, Annual Meeting, June 20-21.

Two methods for detecting the lupus serum factor that reacts with nuclei and nucleoprotein are reported. The first employs fluorescent antihuman globulin. The fluorescent spots are visualized grossly with UV light. The second employs nucleohistone or artificial complexes of DNA and histone coated on glass surfaces and subsequently reacted with antiglobulin labeled with iodine 131. The conditions of the reaction are outlined and results to be expected are noted.

270

Friou, G.J. 1958. The significance of the lupus globulin nucleoprotein reaction. Ann. Intern. Med. 49:866-875.

Studies with the fluorescent antibody technique have shown that sera from patients with disseminated lupus erythematosus contain a globulin factor with a marked affinity for nuclei. With the use of this method, the nuclear component involved in the reaction was found to be in the nucleoprotein fraction of the cell nucleus. Evidence was presented indicating that this reaction is an essential step in the lupus erythematosus phenomenon. A clinical survey was made in which the affinity of human sera for spots of nucleoprotein was tested with fluorescent antiglobulin. The factor was consistently demonstrable in serum from lupus patients, and in low titer in serum from occasional patients with rheumatoid arthritis and other related diseases. Results of tests in which the factor was detected with antiglobulin labeled with iodine 131 indicated that this method might be used to measure the factor in human sera. The significance of these observations was discussed.

280

Friou, G.J. 1958. Identification of the nuclear component of the interaction of lupus erythematosus globulin and nuclei. *J. Immunol.* 80:476-481.

Using fluorescent antibody, it has been shown that the factor that reacts with nuclei also reacts with deoxyribonucleoprotein, or nucleohistone, from calf or rabbit thymus and with artificial complexes of DNA with histone. A technique for titration of the affinity of sera for nucleohistone has been devised. Use of this method has demonstrated the presence of the factor in all lupus sera tested, regardless of results of the lupus erythematosus, LE, test. By absorption of lupus serum with nucleohistone it was possible to remove the affinity for nucleohistone and the capacity to induce the LE phenomenon, suggesting that the two effects may be due to one factor. The broad species range of the reaction has been confirmed by this improved technique.

290

Friou, G.J. 1958. Clinical application of a test for lupus globulin nucleohistone interaction using fluorescent antibody. *Yale J. Biol. Med.* 31:40-47.

Sera from patients with a variety of human diseases have been tested for presence of a globulin factor that reacts with nucleoprotein. The tests were made, and activity of the factor was titrated, with an adaptation of the fluorescent antibody technique. The factor was found to be characteristic of sera of patients with disseminated lupus erythematosus, especially when present in high titer. It was also present in low titer in occasional sera of patients with disease syndromes known to be closely related to lupus, especially rheumatoid arthritis, and in individuals with chronic biologic false positive serologic tests for syphilis. The possible significance of these findings is discussed and use of this procedure as a diagnostic method is considered.

300

Friou, G.J. 1962. Fluorescent spot test for antinuclear antibodies. *Arth. Rheum.* 5:407-410.

Some time ago we described a procedure for detection of antinuclear antibodies, using dried spots of nucleoprotein on glass slides as antigen and fluorescent antiglobulin as the indicator system. Although the fluorescent antibody technique is used, results may be read by gross inspection with an inexpensive fluorescent light source. Continued use of the method has reinforced our earlier experience and has indicated that results obtained with this method and the difficult but more quantitative methods using anti-globulin labeled with iodine 131 are comparable. In our laboratory the fluorescent spot test has appeared to be especially useful because of the minimal subjective influence in reading the results and its simplicity. Others have had very satisfactory experiences with the procedure and in general have confirmed our findings. Nevertheless, certain details of the procedure must be carefully followed if satisfactory results are to be obtained. Furthermore, we have encountered and largely overcome certain technical problems in the procedure as originally described. Although the method and results obtained remain basically unchanged, these modifications have not been published and the procedure is, therefore, described here in complete detail.

18

310

Friou, G.J.; Finch, S.C.; Detre, K.D. 1957. Nuclear localization of a factor from disseminated lupus serum. Federation Proc. 16:1770:413.

In vitro studies of localization of human serum in normal mouse tissue sections were done with a modified fluorescence technique. Sera from all of a large number of patients with disseminated lupus erythematosus caused nuclear localization of fluorescent anti-human globulin. Many other sera were studied, including those of patients with related diseases, infections, and drug reactions. Of this large group, localization similar to that observed with lupus serum was encountered only with sera from two patients with unusually severe hypersensitive reactions to drugs.

320

Friou, G.J.; Finch, S.C.; Detre, K.D. 1958. Interaction of nuclei and globulin from lupus erythematosus serum demonstrated with fluorescent antibody. J. Immunol. 80:324-329.

Using the fluorescent antibody technique, a globulin component with a marked affinity for nuclei has been demonstrated in sera of patients with disseminated lupus erythematosus. It has been shown that the factor reacts with nuclei of most tissues of a wide range of vertebrate species. Information on the occurrence of the factor in 100 human sera is reported.

330

Gitlin, D.; Craig, J.M.; Janeway, C.A. 1957. Studies on the nature of fibrinoid in the collagen diseases. Amer. J. Pathol. 33:55-57.

Sections of tissues from patients with rheumatoid arthritis, disseminated lupus erythematosus, dermatomyositis, rheumatic fever, subacute and chronic glomerulonephritis, and periarteritis nodosa were studied with fluorescein-labeled rabbit antisera against human fibrin, albumin, and gamma globulin. Suitable controls were used to insure the specificity of the method for the detection of these proteins in sections of human tissues. Replicate sections were studied with phosphotungstic acid hematoxylin, Biebrich scarlet-aniline blue, periodic acid-leukofuchsin-crane G-hematoxylin, and periodic acid-leukofuchsin; these methods ordinarily are considered suitable for the detection of fibrin. Tissue samples from normal placentas and from acutely inflamed appendices were studied also. The data indicate that the fibrinoid material found in the lesions associated with the collagen diseases is, at least in part, fibrin. It was observed also that the conventional staining methods employed were not reliable for revealing fibrin in tissue sections, since they frequently gave negative results for areas in which fibrin could be demonstrated with specific fluorescent antibodies. The significance of these findings is discussed.

340

Goodman, H.C.; Fahey, J.L.; Malmgren, R.A. 1960. Serum factors in lupus erythematosus and other diseases reacting with cell nuclei and nucleoprotein extracts: Electrophoretic, ultracentrifugal, and chromatographic studies. *J. Clin. Invest.* 39:1595-1605.

Multiple serum factors in lupus erythematosus, LE, patients reacted with parts of cell nuclei. These anti-cell nucleus factors were detected by various tests including the fluorescent antoglobulin test, and all were in the gamma globulins. LE factor and antithymus nucleoprotein were in the 6.6S gamma globulins. Antihuman liver nucleoprotein was principally in the 18S gamma globulin with a little in the 6.6S. The same was true of anti-cell nuclei activity. Anion exchange chromatography was helpful in separating and identifying the gamma globulins.

350

Goodman, H.C.; Malmgren, R.A.; Fahey, J.L.; Brecher, G. 1959. Separation of factors in lupus erythematosus serum reacting with components of cell nuclei. *Lancet* 2:382-383.

Lupus erythematosus sera may contain several factors reacting with components of cell nuclei. These serum factors can be separated chromatographically into at least two distinct fractions. The evidence suggests that certain antinuclei activities reside in the 6.6(7)S gamma globulins and others within the 18S gamma macroglobulins.

360

Hijmans, W.; Doniach, D.; Roitt, I.M.; Holborow, E.J. 1961. Serological overlap between lupus erythematosus, rheumatoid arthritis, and thyroid autoimmune disease. *Brit. Med. J.* 2:909-914.

Patients with the subject diseases were tested for autoantibody production. Antinuclear factor and thyroid antibody tests were done by FA. Results indicated that the autoimmune diseases form a broad overlapping spectrum with antigen disturbances at one end and antibody production disturbances at the other.

370

Hijmans, W.; Schuit, H.R.E.; Leeuw, B.; Moller, H.F. 1962. The diagnostic value of antinuclear factors demonstrated by means of fluorescent antibodies. *Ned. Tijdschr. Geneesk.* 106:2097-2101. In Dutch.

FA has made it possible to demonstrate antinuclear factors present in most systemic lupus erythematosus cases. Positive tests are common also in rheumatoid arthritis but rare in normal persons. FA can be used as a preliminary test and is easy to perform.

380

Holborow, E.J. 1960. Serum antinuclear factor and autoimmunity. Proc. Roy. Soc. Med. 53:625-627.

A serum factor present in SLE is also found in discoid lupus, rheumatoid arthritis, Still's disease, and Hashimoto disease. In SLE abnormal gamma globulins are produced, perhaps because of mutational change in and stimulation of antibody-forming cells. The overlap of antinuclear factor into the disease groups described indicates a wider occurrence of a similar departure from normal immunological status.

390

Holborow, E.J.; Weir, D.M. 1959. Histone: An essential component for the lupus erythematosus antinuclear reaction. Lancet 1:809-810.

This work was based on the previously shown phenomenon that the sera of patients with systemic lupus erythematosus contain globulins with affinity for cell nuclei. Treating frozen sections of guinea pig testis and epididymis with normal human serum and others with sera known to contain antinuclear factor, followed by antiglobulin fluorescein conjugate, demonstrated localization in immature cell nuclei but not in sperm heads. Since mature sperm heads lack an essential component for the reaction, and previous work has demonstrated the transformation of nucleohistone into a protamine substance at the end of maturation, it is postulated that the essential factor for the antinuclear reaction is histone.

400

Holborow, E.J.; Weir, D.M.; Johnson, G.D. 1957. A serum factor in lupus erythematosus with affinity for tissue nuclei. Brit. Med. J. 2:732-734.

We have shown that sera giving a positive lupus erythematosus cell test contain a globulin factor with affinity for tissue nuclei.

410

Holman, H.R.; Kunkel, H.G. 1957. Affinity between the lupus erythematosus serum factor and cell nuclei and nucleoprotein. Science 126:162-163.

The data suggest that the LE serum factor has an affinity for nuclear nucleoprotein and that deoxyribonucleic acid is involved in the bond. The fact that the LE serum factor is a gamma globulin that appears to react with antiserum to γ or α_1 gamma globulin suggests that the factor may be an antibody. This possibility merits further investigation, with particular reference to the question of whether or not the LE factor could be an auto-antibody to nucleoprotein or deoxyribonucleic acid.

420

Kaplan, M.H. 1959. Autoantibodies to heart tissue in the sera of certain patients with rheumatic fever. Federation Proc. 18:2263:576.

Sera of patients with rheumatic fever frequently exhibit immunofluorescent staining of tissue sections of normal human heart. This staining reaction is due to the presence in such sera of either of two reactive gamma globulins. The first has been found only in the sera of certain patients with rheumatic heart disease and gives a staining pattern with characteristic juxta-myofibrillar and subsarcolemmal distribution. This staining capacity may be specifically absorbed with heart tissue homogenate; alcoholic and saline extracts of heart have been ineffective. In the second type of reaction, given by rheumatic and certain other pathologic sera, the staining of myofibers is similar but not identical to that described above. Further, this staining capacity is associated with complement-fixing and flocculating activity of the sera with alcoholic extracts of normal heart. One stimulus for the production of the first type of autoantibody may be cardiac surgery itself. The pattern of sarcoplasmic staining given by this autoantibody is similar to the pattern of distribution of deposited gamma globulin with myofibers of some rheumatic specimens. This observation suggests that in these patients autoantibody to a myofiber constituent may participate in the pathogenesis of rheumatic heart disease.

430

Kaplan, M.H.; Craig, J.M. 1958. Production of cardiac lesions in rabbits immunized with heterologous heart tissue. Federation Proc. 17:2030:520.

Immunization of rabbits with homogenates of beef or rat heart stimulated antibodies reactive with normal rabbit heart tissue, as demonstrated by immunofluorescence and complement-fixation. The antigen has been identified as an alcohol-soluble organ-specific constituent of the sarcoplasm of striated muscle. It may be differentiated by cross-absorption tests from cardiolipin antigen. Rabbits given repeated injections of beef or rat heart homogenate incorporated in either alumina gel or Freund adjuvant develop small focal cardiac lesions characterized by myofiber necrosis and or interstitial inflammation. Rarely, an extensive myocardial fibrosis has been observed. The results of one experiment were as follows: Cardiac lesions were noted in 8 of 8 animals injected with rat heart, 7 of 9 animals given beef heart, and in 3 of 13 control rabbits injected with human gamma globulin. Myofiber necrosis was noted in 12 of the 17 experimental animals and in one of the 13 controls. In 7 of 15 experimental animals, bound gamma globulin could be detected within the sarcoplasms of myofibers, although all of these 15 rabbits showed elevated levels of circulating antibody. Myocardial cells are resistant to penetration by antibody to the sarcoplasmic antigen. Resistance may represent a limiting factor in pathogenesis of cardiac lesions.

440

Kaplan, M.H.; Dallenbach, F.D. 1961. Immunologic studies of heart tissue: III. Occurrence of bound gamma globulin in auricular appendages from rheumatic hearts: Relationship to certain histopathologic features of rheumatic heart disease. *J. Exp. Med.* 113:1-16.

Using FA, deposits of bound gamma globulin, as determined in unfixed washed sections of auricular appendages from rheumatic hearts, were noted in 18 per cent of 100 specimens studied. Such deposits were observed in myofibers, sarclemma, interstitial connective tissue, and vessel walls. Albumin and fibrin were generally absent. Control hearts from normal and pathologic material, including postmortem and biopsied specimens, did not reveal such deposits. These various tissue sites that contained bound gamma globulin frequently exhibited evidence of alteration, as indicated both by enhanced affinity for eosin and by strongly positive reaction with the periodic acid-Schiff reagent, and appeared comparable in some cases to fibrinoid. Bound gamma globulin was not observed in cellular or stromal components of Aschoff lesions, nor was the occurrence of Aschoff lesions correlated with presence of bound gamma globulin. It is suggested that deposition of gamma globulin and the eosinophilic alteration associated with such deposition are related to certain pathologic changes of rheumatic heart disease.

450

Kaplan, M.H.; Meyserian, M. 1962. Immunologic studies of heart tissue: V. Antigens related to heart tissue revealed by cross-reaction of rabbit antisera to heterologous heart. *J. Immunol.* 88:450-461.

Injection of rabbits with whole homogenates of heterologous heart from beef or rat, incorporated in aluminum hydroxide gel adjuvant, induced antibodies strongly reactive with saline extracts of normal rabbit heart by precipitation and complement fixation. These antisera were not reactive in precipitation tests with extracts of other organs except for occasional weak reactions with skeletal muscle. Absorption tests with skeletal muscle indicated antigenic specificity for heart tissue. In two-dimensional complement fixation tests with antisera to heterologous heart, rabbit heart extract showed far higher titers of reactive antigen than extracts of other rabbit organs. This heart-related antigenic material was sedimentable at high speed and its cellular localization was correlated with material in myocardial cells distributed between myofibrils. These observations are consistent with origin of the above antigen or antigens from sarcoplasmic reticulin or mitochondria. Separate heterogenetic organ-related antigenic activity of rabbit skeletal muscle was demonstrated.

460

Kaplan, M.H.; Meyserian, M. 1962. An immunological cross-reaction between group A streptococcal cells and human heart tissue. *Lancet* 1:706-710.

Widespread deposits of bound gamma globulin were observed in the ventricular muscle of a patient who died of acute rheumatic fever with severe cardiac failure. The possible occurrence of an antigen in group A streptococci immunologically cross-reactive with heart tissue was investigated. Antisera prepared in rabbits against group A streptococcal cell walls exhibited immunological reaction with human heart tissue by immunofluorescence

and complement fixation. The reactant in human heart was distributed in cardiac myofibrils and in smooth-muscle elements of arteries, arterioles, and endocardium. Serological reaction was abolished by absorption of antisera with streptococcal cells or cell walls, but not by cell protoplasm or protoplast membranes. Cross-reactive antigen was associated with M protein, but not with A carbohydrate. Immunization of rabbits with such active fractions elicited antibodies reactive with human heart. In rheumatic fever an autoimmune reaction to a constituent of myofiber and smooth muscle may be induced by exposure to a cross-reactive antigen of group A streptococci.

470

Kaplan, M.H.; Meyserian, M.; Kushner, I. 1961. Immunologic studies of heart tissue: IV. Serologic reactions with human heart tissue as revealed by immunofluorescent methods: Isoimmune, Wassermann, and autoimmune reactions. *J. Exp. Med.* 113:17-36.

In the heart, blood group substances A and B were distributed in capillary walls, vascular endothelium, and interstitial connective tissue. Isoimmune sera reactions against substances A and B were infrequent because of limited sensitivity of FA. Wassermann antibody was reactive with parts of myocardial sarcoplasm, especially cardiolipin. Wassermann-positive sera absorbed with beef cardiolipin reacted with other constituents of myofiber sarcoplasm. Sera of patients with rheumatic fever, rheumatic heart disease, rheumatoid arthritis, disseminated lupus, and liver disease frequently reacted with constituents of myofiber sarcoplasm. These factors were not Wassermann antibody. Three FA patterns could be differentiated by distribution. Reactants could be extracted with ethanol and methanol but not with acetone. FA-reactive sera frequently gave positive flocculation and complement fixation tests with alcohol extracts of human heart. FA tests best correlated with flocculations. Serologic reactions with homologous or autologous heart were frequent with sera from patients approximately two weeks following cardiac surgery, cardiac surgery, or acute myocardial infarction.

480

Kaplan, M.H.; Vaughan, J.H. 1959. Reaction of rheumatoid sera with synovial tissue as revealed by fluorescent antibody studies. *Arth. Rheum.* 2:356-358.

Immunofluorescent studies revealed that gamma globulin may become fixed in synovial tissue during nonspecific inflammation. This gamma globulin is also reactive with rheumatoid factor. Both rheumatoid factor and reactant gamma globulin may occur bound in the tissue.

490

Kritzman, J.; Kunkel, H.G.; McCarthy, J.; Mellors, R.C. 1961. Studies of a Waldenstrom-type macroglobulin with rheumatoid factor properties. *J. Lab. Clin. Med.* 57:905-917.

A case of typical Waldenstrom macroglobulinemia is described in which the pathologic macroglobulin showed biologic activity similar to rheumatoid factors. The protein showed physical, chemical, and antigenic properties very similar to those of the macroglobulins seen in other cases of Waldenstrom activity. The resemblance to rheumatoid factors was striking in certain of the serological tests. Difference in pH optima was

observed that caused negative tests where alkaline buffers were employed. The fluorescent labeling technique demonstrated the site of origin of the pathologic protein.

500

Krooth, R.S.; Tobie, J.E.; Tjio, J.H.; Goodman, H.C. 1961. Reaction of human sera with mammalian chromosomes shown by fluorescent antibody technique. *Science* 134:284-286.

Certain human sera, including sera from five patients with lupus erythematosus, react with mammalian chromosomes. If chromosomal preparations are exposed first to the serum and then to horse antihuman globulin conjugated with fluorescein, the chromosomes will fluoresce. Sera having this activity appear to react with all the chromosomes of the cell.

510

Kunkel, H.G.; Holman, H.R.; Deicher, H.R.G. 1959. Multiple autoantibodies to cell constituents in systemic lupus erythematosus, p. 429-449. In *Ciba Foundation Symposium on Cellular Aspects of Immunity*. Little, Brown, and Co., Boston.

The sera of patients with lupus erythematosus contain a large number of factors that react with different constituents of cells of human and bovine origin. At least six such factors have been identified, and there probably are many more. The factors reacting with DNA and nucleoprotein are of particular interest, and these have been isolated. They possess and appear to represent autoantibodies. At least two cytoplasmic factors are also present. Complement-fixation reactions with different isolated cell constituents have revealed the presence of a strong genetic factor. Few clues are available on the apparent general breakdown of self-recognition mechanisms in immune processes in this disease.

520

Lee, S.L.; Meiselas, L.E.; Zingale, S.B.; Richman, S.M. 1961. Effect of 6-mercaptopurine administration on antibody production and clinical course of systemic lupus erythematosus: Report of a case. *Arth. Rheum.* 4:56-63.

A patient with systemic erythematosus in clinical relapse was treated with 6-mercaptopurine in a dosage of 2.5 mg per kilogram of body weight per day. An initial apparently favorable clinical response was followed by toxic and, later, septic manifestations. Definite depression of gamma globulin and antibody production was observed in this patient following 6-MP therapy. However, no inhibition of primary response to stimulation with Brucella antigen could be demonstrated.

530

Mandema, E.; Pollack, V.E.; Kark, R.M.; Rezaian, J. 1961. Quantitative observations on antinuclear factors in systemic lupus erythematosus. *J. Lab. Clin. Med.* 58: 337-352.

This study was designed to investigate the relationship between clinical phenomena and the titer of antinuclear factors in the sera of patients with systemic lupus erythematosus. Antinuclear factors were titrated with FA. Both rat kidney tissue and human buccal mucosal cells served as the source of cell nuclei. The technique was more sensitive with human buccal mucosal cells. At a 1:4 dilution or more, antinuclear factors were demonstrated in 131 of 151 patients with systemic lupus erythematosus but in only one of 90 control sera, diluted 1:4, from healthy subjects and from patients with diseases unrelated to systemic lupus erythematosus. They were demonstrated in 24 of 50 relatives of patients with systemic lupus erythematosus, indicating a tendency to inheritance. In patients with systemic lupus erythematosus a positive relationship was found between the titer of antinuclear factors and clinical activity of the disease. Adequate control of the disease with Prednisone led to a fall in the titer of anti-nuclear factors, but clinical exacerbations of systemic lupus erythematosus were accompanied by a titer rise.

540

McCormick, J.N. 1962. Use of fluorescein-labeled rheumatoid factor for locating sites of antibody fixation in tissues. *Nature* 194:302-303.

The macroglobulin component of the serum of patients with rheumatoid arthritis, rheumatoid factor, was conjugated with fluorescein and used as an immunological reagent. Rheumatoid factor has an affinity for specifically bound antibody. This reagent was used to demonstrate specifically bound antibody in tissue sections.

550

McKenna, J.L.; Pisciotta, A.V. 1962. Fluorescence of megakaryocytes in idiopathic thrombocytopenic purpura stained with fluorescent antiglobulin serum. *Blood* 19:664-675.

Direct and indirect FA techniques were successful. Fluorescent megakaryocytes were seen in bone marrow of chronic but not acute idiopathic thrombocytopenic purpura patients. Normal megakaryocytes were made fluorescent by preincubation with sera from chronic patients and from a woman whose child had neonatal thrombocytopenia. The same was noted with systemic lupus erythematosus patients. Findings indicated an adhering humoral substance for megakaryocytes of possible etiologic significance.

560

Meiselas, L.E.; Zingale, S.B.; Lee, S.L.; Richman, S.M.; Siegel, M. 1961. Antibody production in rheumatic diseases: The effect of Brucella antigen. J. Clin. Invest. 49:1872-1881.

Forty-one patients with various rheumatic diseases and 27 control patients were inoculated with Brucella vaccine. As a group the patients exhibited a significantly greater rise in anti-Brucella agglutinins compared with the controls. Some overlap was present in both groups. Alterations were noted in other antibody systems, Coombs anti-red cells, anti-thyroglobulin, and possibly in the influenza antibody and rheumatoid factor after this primary stimulation in some of the patients with rheumatic diseases, but no titers for these antibodies were noted in the control patients. The effect of Brucella antigen in these patients may be related to the damage that this organism can produce on mesenchymal tissue.

570

Mellors, R.C.; Heimer, R.; Corcos, J.; Korngold, L. 1959. Cellular origin of rheumatoid factor. J. Exp. Med. 110:875-886.

Fluorescein-labeled human gamma globulin reacted in precipitin-type tests with sera of individuals with rheumatoid arthritis. This reagent was also highly reactive and specific for the localization of rheumatoid factor in frozen sections of synovial membranes, lymph nodes, and subcutaneous nodules. In synovial membranes from patients with active rheumatoid arthritis, rheumatoid factor was present in the cytoplasm of plasma cells only. The appearance of the cytoplasm and the occasional presence nearby of extracellular particles suggested a secretory process. Some plasma cells contained a 7S and or 19S gamma globulin and many lacked detectable gamma globulin. In lymph nodes from a patient with active rheumatoid arthritis, rheumatoid factor was present in approximately one in ten germinal centers as well as in internodular plasma cells. Rheumatoid factor was in cytoplasm and protoplasmic processes of the germinal-center cells only. 7S and or 19S globulin was demonstrated in approximately eight of ten germinal centers in these lymph nodes. Plasma cells with rheumatoid factor were also seen in rheumatoid subcutaneous nodules. Normal and pathological control material did not contain rheumatoid factor. Staining was blocked by pretreatment of sections with unlabeled human gamma globulin or with rabbit antiserum against 19S human gamma globulin.

580

Mellors, R.C.; Nowoslawski, A.; Korngold, L. 1961. Rheumatoid arthritis and the cellular origin of rheumatoid factors. Amer. J. Pathol. 39:533-546.

Specific reactants were used for the microscopic detection of rheumatoid factor *in situ* in tissue sections: rhodamine-labeled aggregated human gamma globulin and fluorescein-labeled rabbit immune complex. Frozen sections of synovial membranes and lymph nodes obtained from patients with active rheumatoid arthritis were treated with the fluorescent reactants in simultaneous, sequential, and separate staining procedures. Tissue rheumatoid factor has been demonstrated in each of the 19 specimens of active rheumatoid synovitis examined and in 15 lymph nodes obtained from patients with active rheumatoid arthritis. The results obtained with the simultaneous and sequential staining procedures

indicated that there were at least two cellular rheumatoid factors: one, generally the more abundant, was detected only with fluorescent aggregate; the other was demonstrable with fluorescent-immune complex as well as with fluorescent aggregate. Some plasma cells formed one rheumatoid factor, some formed a second, and others formed both. Germinal-center cells synthesized either one or the other but not both in the same center. Plasma cell origin, as well as known immunochemical properties, of rheumatoid factor suggests an antibody-like nature and function. Cellular rheumatoid factor was not detectable in any of the 22 synovial specimens obtained from patients with other forms of synovitis and arthritis or in controls.

590

Mellors, R.C.; Nowoslawski, A.; Korngold, L.; Sengson, B.L. 1961. Rheumatoid factor and the pathogenesis of rheumatoid arthritis. *J. Exp. Med.* 113:475-496.

Two fluorescent reactants have been prepared for the detection of rheumatoid factor in tissue sections: fluorescein-labeled rabbit immune complex and fluorescent-aggregated human gamma globulin. Plasma cells in the synovial membrane and germinal-center cells and internodular plasma cells in lymph nodes are the sites of origin of rheumatoid factor in active rheumatoid arthritis. Plasma cells and germinal-center cells that form rheumatoid factor detectable with fluorescent immune complex are less numerous than those that contain factor demonstrable with fluorescent aggregate. Plasma cells and germinal-center cells that contain macroglobulin, 19S human gamma globulin, detectable with fluorescent antibody are more abundant than those containing rheumatoid factor. Macroglobulin and rheumatoid factor are almost exclusively in cytoplasm. Normal and pathological synovial and capsular tissues, lymph nodes, and connective tissues obtained from individuals without rheumatoid arthritis are not FA-stained. The cellular origin, as well as certain chemical and immunological attributes, of rheumatoid factor suggests an antibody-like nature and function. Lesion-associated protein precipitates of the rheumatoid factor, antigen complex are localized in amyloid depositions in kidney and spleen.

600

Mellors, R.C.; Ortega, L.G. 1956. Analytical pathology: III. New observations on the pathogenesis of glomerulonephritis, lipid nephrosis, periarteritis nodosa, and secondary amyloidosis in man. *Amer. J. Pathol.* 32:455-499.

This study shows histopathologic application of a microfluorescence method for demonstrating the histologic sites of localized human gamma globulins. It is established that gamma globulins are localized in the active glomerular lesions in lipid nephrosis of childhood and glomerulonephritis occurring in man at all ages. Gamma globulins are localized in the glomerular and the arterial lesions in periarteritis nodosa and in glomerular lesions in renal amyloidosis. By demonstrating that antibody proteins are localized in the sites of the lesions, the observations fulfill a requirement essential for the immunoallergic pathogenesis of these several diseases of man. Localized gamma globulins are, at least in part, antibodies against antigens that have localized in the protective sites of the lesions. This investigation suggests that the pathogenesis of glomerulonephritis initiated in eclampsia of pregnancy, rheumatic fever, rheumatoid arthritis, disseminated lupus erythematosus, and other so-called collagen diseases, will be further elucidated by the analytical procedure used in this study.

610

Mellors, R.C.; Ortega, L.G.; Holman, H.R. 1957. Role of gamma globulins in pathogenesis of renal lesions in systemic lupus erythematosus and chronic membranous glomerulonephritis, with an observation on the lupus erythematosus cell reaction. *J. Exp. Med.* 106:191-202.

By using fluorescent antibody to localize gamma globulins, we have observed in systemic lupus erythematosus that gamma globulins were localized in the thickened walls, the wire loop lesions, and the so-called hyaline thrombi in glomeruli. Localizations of gamma globulins were correlated with the accentuated eosinophilia of the glomeruli, or with PAS-positive areas; gamma globulins were localized rarely in large cytoplasmic granules, in tubular epithelium, or in glomerular capsular crescents, tubular protein casts, or inflammatory cells, either immature or mature plasma cells. In nephrotic glomerulonephritis gamma globulins were localized in the glomerular basement membrane and appertaining structures in chronic membranous glomerulonephritis, and in the altered mesangium in chronic lobular glomerulonephritis. In the tubular protein casts gamma globulins were present in a lesser concentration and other serum proteins in a greater concentration than found in the glomeruli. In positive lupus erythematosus preparations the nuclei of leukocytes were the sites of localization of gamma globulin.

620

Mellors, R.C.; Ortega, L.G.; Noyes, W.F.; Holman, H.R. 1957. Further pathogenic studies of diseases of unknown etiology, with particular reference to disseminated lupus erythematosus and Boeck sarcoid. *Amer. J. Pathol.* 33:613-614.

In chronic membranous glomerulonephritis, gamma globulins, but not other serum proteins, are present in the thickened glomerular basement membranes. In disseminated lupus erythematosus, gamma globulins are localized strikingly in the wire loop lesions and in the thickened basement membranes of involved glomeruli, occasionally in focal areas of the Bowman capsule, in some plasma cells in the interstitium, and rarely in sites of hyaline droplet degeneration in tubular epithelium. In disseminated lupus erythematosus, C-reactive protein is focally localized in the lymph nodes immediately surrounding the epithelioid granulomas in which are present hyaline materials and plasma, lymphoid and reticular cells. Findings are viewed relative to the statement by Teilum.

630

Movat, H.Z. 1960. Pathology and pathogenesis of the diffuse collagen diseases: III. Pathogenesis of the collagen disease. *Can. Med. Ass. J.* 85:797-803.

Speaking of the collagen diseases in general, it seems reasonable to conclude, first, that fibrinoid, the characteristic change in these diseases, is not an intrinsic alteration of connective tissue, but the result of exudation, precipitation, and inspissation of plasma proteins; secondly, that there is considerable evidence, morphological, immunological, and experimental, that the lesions of the diffuse collagen diseases are related to an immune mechanism. Only in rheumatic fever is there conclusive evidence that an exogenous agent, namely the beta-hemolytic Streptococcus, is implicated in the development of the disease. In some cases of polyarteritis nodosa, exogenous agents such as sulphonamide, iodine, or penicillin, have been identified. In the other conditions of

the group no exogenous agent is known, and the currently accepted view is that they represent an altered state of reactivity of the tissues, which involves perhaps the production of antibodies against endogenous tissue components.

640

Parker, R.L.; Schmid, F.R. 1962. Phagocytosis of particulate complexes of gamma globulin and rheumatoid factor. *J. Immunol.* 88:519-525.

Precipitates formed by the interaction of rheumatoid sera and fluorescent gamma globulin were ingested by viable leukocytes. This reaction required preliminary heating of the gamma globulin. The results suggest that the reactive portion of the gamma globulin is in the form of aggregates. The phenomenon of phagocytosis demonstrates a new biologic property of rheumatoid factor and gamma globulin precipitates and illustrates another resemblance of these precipitates to antigen-antibody complexes.

650

Paronetto, F.; Strauss, L. 1962. Immunocytochemical observations in periarteritis nodosa. *Ann. Intern. Med.* 56:289-296.

A case of polyarteritis offered an opportunity for immunological and immunocytochemical investigations. Gamma globulin, fibrinogen, and albumin were demonstrated in acute vascular and renal lesions. These findings are interpreted as imbibition of the damaged tissues with plasma proteins. Immunohistochemical findings in the spleen and lymph nodes and some serological reactions suggest an immunological component. Gamma globulin was found in cells of the spleen, lymph nodes, and kidneys. Cells in the spleen, characteristic of plasma cells, contained macroglobulin. Positive staining with aggregated fluorescein-labeled human gamma globulin suggests that they contain rheumatoid factor. This finds support in vascular wall antigens. It is unlikely that autospecific antigen-antibody reaction caused the arteritic lesions.

660

Peterson, W.C., Jr.; Gokcen, M. 1962. Antinuclear factors in chronic discoid lupus erythematosus. *Arch. Derm.* 86:783-787.

Sera from 16 women and 18 men with chronic discoid lupus erythematosus were tested for antinuclear activity. Nine women were positive and five weakly positive. Eight men were positive, and one was weakly positive. Speckled nuclear fluorescence was observed in four of each group. The LE cell test was negative in all cases.

670

Pollack, V.E.; Mandema, E.; Kark, R.M. 1960. Antinuclear factors in the serum of relatives of patients with systemic lupus erythematosus. *Lancet* 2:1061-1063.

FA was used to study antinuclear factors in the serum of relatives of systemic lupus erythematosus victims. The etiology of SLE is unknown, but the antigen-antibody irregularities seem to indicate disarrangement in the system regulating immunological homostasis. This study indicates a possible hereditary basis for this derangement.

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680

Rapp, F. 1962. Localization of serum antinuclear factors in tissue culture. Federation Proc. 21:15.

Cells grown in culture were used to detect antibodies in human serum against nuclear components by the indirect immunofluorescent technique. Results with 150 sera correlated well with those obtained by complement fixation against calf thymus nuclei and with the detection of the lupus erythematosus cell, but the immunofluorescent technique was slightly more sensitive than the other methods. Some sera reacted primarily with DNA-associated molecules, other sera combined with antigens near the nuclear membrane, still others reacted with both sites. Chromosomes of cells in all stages of mitosis were reactive. HeLa cells were routinely employed, but cells derived from human embryonic heart, chicken embryos, adult rabbit kidney, and fetal rabbit skin possess reactive sites. Antinuclear antibodies did not penetrate the viable cell and did not prevent growth and multiplication of living cells in culture. It is concluded that the test described is a sensitive one for the detection of antinuclear antibodies and allows precise localization of the reactive sites in the cell.

690

Rapp, F. 1962. Localization of antinuclear factors from lupus erythematosus sera in tissue culture. J. Immunol. 88:732-740.

Cells grown in culture have been used to detect antibodies in lupus erythematosus, LE, sera against nuclear components by means of the immunofluorescent antibody technique. This technique was shown to correlate well with complement fixation and with the detection of LE cells. Localization of the reaction on the chromosomes indicates that some sera react primarily with the DNA or DNA-associated molecules. This reaction occurs in cells in various stages of mitosis as well as in the interphase cell. Other sera were found to react with antigens near the nuclear membrane. Antinuclear antibodies do not appear to penetrate the viable HeLa cell and do not prevent growth and multiplication of living cells in culture. It is suggested that the combination of tissue culture and immunofluorescence is a precise and sensitive method for the detection of antinuclear antibodies.

700

Rothbard, S.; Watson, R.F. 1961. Antigenicity of rat collagen: Demonstration of antibody to rat collagen in the renal glomeruli of rats by fluorescence microscopy. J. Exp. Med. 113:1041-1051.

Rabbit serum or globulin containing antibody to rat collagen, when injected intravenously or intracardially into normal or adjuvant-prepared rats, becomes fixed in the basement membranes of renal glomeruli and, to a slight extent, of the tubules. This antibody can be identified in tissue sections by the yellow-green fluorescence occurring where the rabbit globulin, associated with the fixed collagen antibody, has reacted with fluorescein-conjugated antirabbit globulin from ducks. The reaction of the antibody to rat collagen with its antigen in the kidney is a primary factor in the production of the renal glomerular injury that occurs in rats prepared with adjuvant. The antibody to rat collagen remains in the kidney as long as 92 days and has been detected

as early as 45 minutes after injection. This series of immunologic tests indicates that the anti-collagen serum reacts with its homologous antigen, presumably collagen, in the basement membranes of renal glomeruli and tubules, and that specific antibody can be used to identify collagen in other tissues of the animal body.

710

Rothbard, S.; Watson, R.F. 1962. Demonstration of injected antibody to collagen in rat tissues by fluorescence microscopy. *Arth. Rheum.* 5:119.

Rabbit serum containing antibody to rat collagen, injected into normal or adjuvant-prepared rats, becomes fixed at the site of its specific antigen. In tissue sections, this antibody can then be identified by fluorescence where the rabbit globulin, associated with the fixed collagen antibody, has reacted with fluorescein-conjugated anti-rabbit globulin from ducks. Antibody to collagen was first demonstrated in the basement membranes of renal glomeruli and, to a lesser extent, of the tubules. It has also been found in the sarcolemma of cardiac and striated muscle; epithelial basement membranes of lung, liver, and intestine; capsules, trabeculae, and fine reticulin framework of lymph node, spleen, and adrenal; adventitia of the aorta; joint synovia; scleral coat of the eye; and connective tissue of the choroid plexus and peripheral nerve. Only in the renal glomeruli of rats prepared with adjuvant did antibody to rat collagen produce any apparent injury. The antibody remained in the tissues as long as 92 days and was detected as early as 45 minutes after injection. Appropriate controls confirmed the specificity of the antibody localization. The reaction of the anti-collagen serum with its homologous antigen in the basement membranes of the renal glomeruli is a primary factor in the glomerular injury that occurs in the rats prepared with adjuvant. This specific antibody can be used to identify collagen in other tissues.

720

Rothbard, S.; Watson, R.F. 1962. Antigenicity of rat collagen: Distribution of antibody to rat collagen injected into rats. *J. Exp. Med.* 116:337-346.

Antibody to rat collagen, prepared in rabbits and injected into the circulation of normal or adjuvant-prepared rats, becomes fixed to its antigen and can then be identified in tissue sections by fluorescence after application of fluorescein-conjugated antirabbit globulin. In heart, lung, liver, spleen, adrenal, kidney, jejunum, lymph node, thymus, joint synovia, peripheral nerve, aorta, skeletal muscle, eye, and brain, antibody was found at all sites where collagen and reticulin are normally present but, except for the kidneys of the adjuvant-prepared rats, no pathological abnormalities were demonstrated. It was not found within cells. Specific fluorescence was absent from tissues of rats injected with normal rabbit serum or rabbit antifish collagen serum or rabbit antirat collagen serum after absorption with rat collagen, but was present when the antirat collagen serum had been absorbed with fish collagen. The reaction could be blocked by pretreatment of sections with unlabeled antirabbit globulin and did not occur with heterologous labeled antiduck globulin. After serial treatment in vitro with homologous antibody to collagen and conjugated antirabbit globulin, purified reconstituted collagen fibers showed the same fluorescence as fibers in the tissues. Antibody to rat collagen is directed toward an antigen present in both collagen and reticulin.

730

Seegal, B.C. 1962. The value of the fluorescent antibody technique in the study of chronic disease. *J. Chron. Dis.* 15:935-940.

This review article places emphasis on the chronic diseases of various etiologies. Included are discussions on microorganism-related disease, connective tissue disorders such as rheumatoid arthritis and lupus erythematosus, endocrinological problems as in Hashimoto disease, nerve demyelination mechanisms, and tumor factors such as viruses and abnormal antigens. Studies of myasthenia gravis, kidney disease, and rheumatic fever receive comment. Both diagnostic applications and mechanism of pathogenesis studies are discussed.

740

Shurin, S.F.; Yavorovskaya, B.Ye.; Lozovoi, V.P. 1961. Detection of virus isolated from patients with rheumatic fever in fibroblast culture by means of labeled fluorescent antiserum. *Probl. Virol. USSR* 6:292-296.

In infection of human fibroblast cultures with a virus isolated from patients with rheumatism, the virus can be visually detected with labeled fluorescent antiserum. The virus apparently is localized initially in the nucleus, but as multiplication continues the virus passes from the nucleus to the cytoplasm. The degree of degeneration of the fibroblast probably corresponds to the maximum accumulation of virus on it. Encounter of cell and virus and the start of virus multiplication are not accompanied by visible changes in the morphology of the fibroblast. Strains of virus 401, 403, and 311 have similar antigenic structure.

750

Strauss, A.J.L.; Seegal, B.C.; Hsu, K.C.; Burkholder, P.M.; Nastuk, W.L.; Osserman, K.E. 1960. Immunofluorescence demonstration of a muscle-binding, complement-fixing serum globulin fraction in myasthenia gravis. *Proc. Soc. Exp. Biol. Med.* 105:184-191.

A muscle-binding complement-fixing component was demonstrated by FA in the crude globulin fraction of a pool of serum from 10 patients with myasthenia gravis of recent onset and progressive character. This was not demonstrated in similarly prepared normal serum globulin. Nontagged myasthenic globulin blocked adherence of fluorescein-tagged myasthenic globulin to skeletal muscle striations, but prior treatment with normal serum globulin intensified FA staining. Individual myasthenia gravis sera in the pool also blocked staining. Normal sera did not block adherence of the fluorescein-tagged myasthenic globulin to skeletal muscle. Myasthenia gravis globulin fraction fixed guinea pig complement to human skeletal muscle, by successive treatment of muscle sections with myasthenic globulin, guinea pig complement, and fluorescein-conjugated rabbit anti-guinea pig complement, but normal serum globulin did not. Thirty-one myasthenia gravis sera, 11 normal sera, and 5 sera from other myopathies were tested for CF as above. Thirteen myasthenic sera were CF-positive. No normal sera fixed complement. Serum from one patient with acute dermatomyositis was CF-positive.

760

Taylor, H.E.; Shepherd, W.E. 1960. The immunohistochemical interaction of autologous rheumatoid serum with subcutaneous rheumatoid nodules. *Lab. Invest.* 9:603-611.

Rheumatoid blood serum was conjugated with fluorescein isocyanate. The conjugate gave a specific fluorescence with a component in the area of fibrinoid necrosis of rheumatoid nodules obtained from the same patient. The reaction also occurred with homologous rheumatoid serum conjugate. The conjugates used for staining contained rheumatoid factor, as determined by positive latex tests. It is believed that the tissue reactant is probably a gamma globulin bound to tissue components, and that this reacts with the blood component, probably a rheumatoid factor. This finding lends further support to the hypothesis that the latter may be an autoantibody to the former.

770

Thompson, G.R. 1962. Serum antinuclear factors associated with systemic lupus erythematosus. *Univ. Mich. Med. Bull.* 28:378-387.

Methods for detecting serum ANF were discussed. Modification of the FA technique, using chicken erythrocyte nuclei as antigen, yielded 85 per cent positives in LE cases and 15 per cent in rheumatoid arthritis cases. The procedure is outlined.

780

Vazquez, J.J.; Dixon, F.J. 1956. Studies on the immunohistochemical composition of inflammatory and degenerative lesions. *Amer. J. Pathol.* 32:615.

The fluorescent antibody technique was applied to the study of the gamma globulin and albumin content of tissue lesions in secondary human amyloidosis, experimental rabbit amyloidosis, and some diseases in the so-called collagen group. Specificity of the technique was evaluated. Amyloid deposits in secondary human amyloidosis and in experimentally produced amyloidosis in rabbits stained strongly for the corresponding gamma globulin and weakly for albumin. The kidney in a case of lupus erythematosus showed gamma globulin and relatively little albumin in areas of fibrinoid change in arterioles and glomeruli, as did subcutaneous nodules of rheumatoid arthritis, particularly in areas of fibrinoid change. In rheumatic heart disease, the altered heart valves as well as connective tissue in the myocardium showed the same. In normal tissues there was more staining for albumin. Albumin with less gamma globulin was present in the exudate of a case of acute appendicitis. More gamma globulin than albumin, however, was found in the chronically inflamed tissue of the wall of a tuberculous pulmonary cavity. The significance of the observed concentrations of gamma globulin in the situation described cannot as yet be determined. They may represent localized antibody.

790

Vazquez, J.J.; Dixon, F.J. 1957. Immunohistochemical study of lesions in rheumatic fever, systemic lupus erythematosus, and rheumatoid arthritis. *Lab. Invest.* 6:205-217.

The hearts of patients with active rheumatic carditis, subcutaneous nodules from patients with rheumatoid arthritis, and spleen, kidneys, and LE cells from patients with systemic

lupus erythematosus were studied for their gamma globulin content by the fluorescent antibody technique, using fluorescent antihuman gamma globulin. The lesions of rheumatic fever, systemic lupus erythematosus, and, to a lesser degree, rheumatoid arthritis, showed significant specific concentrations of gamma globulin. Inclusions within LE cells and so-called free bodies in the peripheral blood of patients with systemic lupus erythematosus showed concentrations of gamma globulin. Localization of gamma globulin in lesions of the above-mentioned diseases is a specific, preferential process, the nature of which remains to be determined. These observations are consistent with but not specific for the presence of an antigen-antibody reaction in the tissue lesions.

800

Vazquez, J.J.; Dixon, F.J. 1958. Immunohistochemical analysis of lesions associated with fibrinoid change. A.M.A. Arch. Pathol. 66:504-517.

FA was used in a study of certain diseases associated with fibrinoid change. The diseases were rheumatic fever, systemic lupus erythematosus, thrombotic thrombocytopenic purpura, abruptio placentae with bilateral cortical necrosis, experimental serum sickness, generalized Shwartzman phenomenon, and an active peptic ulcer in which fibrinoid change was present. The following antisera were prepared for conjugation with fluorescein isocyanate: antihuman gamma globulin, antihuman serum albumin, antirabbit gamma globulin, antirabbit serum albumin, antihuman fibrinogen, and antirabbit fibrin. Results of an immunohistochemical analysis of lesions associated with fibrinoid change indicate that such a change is not chemically identical in all cases. Plasma proteins might play a role in the production of the fibrinoid change. These findings point out differences and similarities among some of the diseases studied. The observations, however, emphasize the danger of applying a unifying concept to fibrinoid and to the diseases associated with this change.

810

Waaler, E. 1962. The immunopathology of the collagen diseases. Acta Pathol. Microbiol. Scand. Suppl. 154:29-39.

This is a review of the collagen diseases: rheumatic fever, rheumatoid arthritis, systemic lupus erythematosus, diffuse scleroderma, dermatomyositis, polyarteritis nodosa, and thrombotic thrombocytopenic purpura. Principal discussion revolves around autoimmunity as demonstrated by FA.

820

Weir, D.M.; Holborow, E.J. 1962. Serum antinuclear factor: Laboratory studies. Ann. Rheum. Dis. 21:40-44.

Antinuclear factor, ANF, has been found mainly in the 7S gamma globulin fraction of serum, but in some sera in the macroglobulin also. It is usually removed by absorption with nucleoprotein. Histochemical studies suggest that the reaction between ANF and nuclei requires the presence of both deoxyribonucleic acid and histone, probably in combination. Immunization of guinea pigs with nucleoprotein failed to produce a specific immunological response.

830

Weir, D.M.; Holborow, E.J.; Johnson, G.D. 1961. A clinical study of serum anti-nuclear factor. *Brit. Med. J.* 1:933-937.

Serum antinuclear factor, as shown by fluorescent antibody, has been found in 62 of 63 cases of systemic lupus erythematosus and in more than 10 per cent of cases of discoid lupus erythematosus, rheumatoid arthritis including Still's disease, and liver disease. The nature of the factor is discussed in the light of current concepts of autoimmunity.

840

White, R.G. 1959. Localization of auto-antigens in the thyroid gland by the fluorescent antibody technique. *Exp. Cell Res. Suppl.* 7:263-274.

The use of fluorescein or rhodamine conjugates of the serum globulin of patients with lymphadenoid goiter, or Hashimoto disease, permits the localization in thyroid sections, including those from the same patient, of an auto-antigen with the characteristics of thyroglobulin. This antigen was present in most of the follicles of all thyroids and in the hypertrophic follicular epithelial cells from toxic thyroids. The hypertrophied nuclei of such cells also contain areas of antigen possibly related to nucleoli. Intracellular antigen is particularly prominent in the enlarged nuclei of lymphadenoid goiter. Four of 25 sera from cases of this disease contain an additional factor that localizes to nuclei, and is possibly identical to the serum factor with a similar affinity for nuclei present in most cases of disseminated systemic lupus erythematosus. The localization of these and other antigens is briefly discussed in relation to the pathogenesis of lymphadenoid goiter.

850

White, R.G.; Bass, B.H.; Williams, E. 1961. Lymphadenoid goiter and the syndrome of systemic lupus erythematosus. *Lancet* 1:368-373.

Forty cases of lymphadenoid goiter, or Hashimoto disease, were tested for the presence in the serum of antinuclear factor, using a histological technique with fluorescent antibody. Five cases were positive. In two patients no other disease was present. One patient had signs of systemic lupus erythematosus 2 years after a positive serum reaction for antinuclear factor, and in another the findings also suggested this diagnosis. In the fifth case, rheumatoid arthritis and rheumatic carditis were present. The existence of such a subgroup of cases of lymphadenoid goiter is discussed in relation to the "leak" hypothesis of the pathogenesis of this disease.

860

White, R.G.; Marshall, A.H.E. 1962. The autoimmune response in myasthenia gravis. *Lancet* 2:120-123.

A series of cases is presented. FA was used to demonstrate antibodies against nuclei. A relationship between myasthenia and systemic lupus erythematosus is suggested.

S70

Widelock, D.; Gilbert, G.; Siegel, M.; Lee, S.L. 1961. Fluorescent antibody procedure for lupus erythematosus: Comparative use of nucleated erythrocytes and calf thymus cells. Amer. J. Public Health 51:829-835.

As a screening procedure, the fluorescent technique utilizing calf thymus nuclei is more sensitive than the other tests performed. Eighty-four per cent of the SLE cases were positive, compared with 74 per cent by LE preparation and 37 per cent by the above fluorescent technique. Diseases other than SLE were found to be positive by this technique, mostly in patients with conditions related to the rheumatic diseases. Use of chicken cells as a screening procedure is not advisable. Family members of SLE cases demonstrated a high percentage of positive results with the calf thymus technique and should be tested regularly.

880

Zingale, S.B.; Barry, R.C.; Rivero, H.H.; Alvarez, B.R. 1959. The LE phenomenon: Study of its protein fluorescence response. Medicina Buenos Aires 19:217-226. In Spanish.

Lissamine rhodamine-conjugated serum from SLE patients was used to demonstrate the incorporation of serum protein into nuclei of normal leukocytes and into free or phagocytized LE bodies. This incorporation was not demonstrated with other sera. The hypothesis is supported that during LE there is an incorporation of serum protein into nuclei and into the LE substance.

III. AUTOIMMUNE DISEASE

890

Anderson, J.R.; Gray, K.G.; Peck, J.S.; Buchanan, W.W.; McElhinney, A.J. 1962. Precipitating autoantibodies in the connective tissue diseases. Amer. Rheum. Dis. 21: 360-367.

FA tests were a minor part of this study.

900

Balfour, B.M.; Doniach, D.; Roitt, I.M.; Couchman, K.G. 1961. Fluorescent antibody studies in human thyroiditis: Autoantibodies to an antigen of the thyroid colloid distinct from thyroglobulin. Brit. J. Exp. Pathol. 42:307-316.

The serum from the 5 to 8 per cent of Hashimoto patients that show no antibodies to thyroglobulin or the microsomal antigen stain the colloid of human thyroid sections by Coons sandwich technique and produce a different pattern from that of anti-thyroglobulin. The antigen CA2 was distinguished from thyroid globulin by its sensitivity to formalin fixation, by a radioactive co-precipitation technique, and by absorption studies. Anti-CA2 antibodies are present in most Hashimoto cases but are also found in other thyroid diseases and are not diagnostic of lymphadenoid goiter.

910

Barnett, E.V.; Dumonde, D.C.; Glynn, L.E. 1962. Induction of autoimmunity to adrenal gland. Arth. Rheum. 5:636.

Rabbits and guinea pigs immunized with adrenal homogenates in complete Freund adjuvant developed autoimmunity to adrenal gland. Heterologous adrenal homogenate was more effective than homologous adrenal immunization in inducing autoantibody and autoimmune adrenalitis. Complement-fixing antibody elicited by immunization with heterologous adrenal was directed primarily against the heterologous adrenal, but exhibited cross-reactions for autologous and fetal adrenal. Antibody elicited by immunization of rabbits with homologous adrenal reacted equally with autologous, homologous, or heterologous adrenal. Repeated hyperimmunization with either homologous or heterologous adrenal resulted in antibody with higher titer and with greater cross-reactivities with nonadrenal autologous and fetal organs. By immunofluorescence the rabbit antibody elicited by heterologous adrenal immunization reacted with autologous ovarian interstitial cells, homologous testicular Leydig cells, and autologous adrenal cortex, but not with adrenal medulla, liver, brain, kidney, or sperm. Antibody from rabbits immunized with homologous adrenal reacted in addition with rabbit sperm. Adrenitis, characterized by infiltrations of the adrenal cortex with lymphocytes or polymorphonuclear cells, was found only in those rabbits given heterologous adrenal. Similar lesions, almost exclusively composed of round cells, were found in guinea pigs immunized with either heterologous or homologous adrenal; however, they were more extensive in those animals immunized with heterologous adrenal. The mechanism for the greater effectiveness of immunization with certain heterologous organs in adjuvant in inducing autoimmunity is unknown. However, this effect is similar to the breaking of tolerance to serum protein by immunization with heterologous protein. Alternate possibilities include a greater adjuvant effect with heterologous antigen.

920

Beutner, E.H.; Witebsky, E. 1962. Studies on organ specificity: XIV. Immunofluorescent studies of thyroid-reactive autoantibodies in human sera. *J. Immunol.* 85:462-475.

The interactions between human sera and human and monkey thyroid antigens were studied by fluorescent antibody, complement fixation, and tanned cell hemagglutination. FA demonstrated three types of antithyroid antibodies in human serum, thyroid colloid, thyroid epithelial cytoplasm, and thyroid nuclei. Results from thyroidectomized patients indicated that thyroglobulin occurred in all chronic nonspecific thyroiditis cases.

930

Beutner, E.H.; Witebsky, E.; Gerbasi, J.R. 1959. Organ-specific reactions of thyroid autoantisera from rabbits, dogs, and humans as determined by the fluorescent antibody technic. *Federation Proc.* 18:2192:559.

Cross-reactions of rabbit, dog, and human thyroid autoantibodies were investigated. The indirect staining method was applied, using conjugates of goat antirabbit globulin, rabbit antidog globulin, and rabbit antihuman globulin for staining reactions with rabbit, dog, and human thyroid autoantisera respectively. Three rabbit thyroid autoantisera that gave positive staining reactions with rabbit thyroid sections all stained human thyroid sections; two sera stained dog thyroid sections. Two dog thyroid autoantisera stained rabbit and human as well as dog thyroid sections. Five human thyroiditis sera were examined for their capacity to stain human, rabbit, and dog thyroid sections. Homologous reactions were generally stronger than cross-reactions. Staining reactions were obtained at serum dilutions up to 1:81 as compared with titers of 1:12,800 and more obtained with the tanned cell hemagglutination technique. The staining reactions considered here were all localized in the colloid or at its periphery.

940

Beutner, E.H.; Witebsky, E.; Ricken, D.; Adler, R.H. 1962. Studies on autoantibodies in myasthenia gravis. *J. Amer. Med. Ass.* 182:46-58.

Direct, indirect, and complement FA staining were used to study antibodies to muscle, using sera from myasthenia gravis patients. Two of 10 sera gave strong reactions. Results indicated involvement of autoantibody. The serum factor involved was found in the gamma globulin. Organ specificity of direct and indirect staining was demonstrated for skeletal and cardiac muscle only; no other tissues stained. The antigen involved in complement staining was specific for skeletal muscle tissue. Pros and cons of myasthenia gravis as an autoimmune disease are discussed.

950

Beutner, E.H.; Witebsky, E.; Rose, N.R.; Gerbasi, J.R. 1958. Localization of thyroid and spinal cord autoantibodies by fluorescent antibody technic. *Proc. Soc. Exp. Biol. Med.* 97:712-716.

Sections of normal spinal cords of rabbits were treated with rabbit antisera produced by immunization of rabbits with rabbit spinal cord suspensions; the treated sections were

stained with fluorescein-conjugated goat antiserum to rabbit immune globulin. Selected areas stained suggest the myeline sheath as at least one of the antibody-combining sites. No staining was obtained with normal sera or sera containing thyroid autoantibodies. Sections of thyroid glands of normal rabbits were exposed to the action of rabbit sera containing rabbit thyroid autoantibodies and stained as above. The colloid extending into adjoining epithelial layers was stained positively. Interstitial tissue remained basically unstained. No positive staining reactions were obtained with normal rabbit serum or rabbit serum containing brain autoantibodies. With thyroid autoantibodies, the positive staining reactions and antibody titer were roughly parallel. However, rabbit autoantisera failed to give staining reactions when diluted more than ninefold. It is not yet possible in studies with spinal cord autoantisera to make a statement regarding appearance of positive staining reactions and presence of demonstrable circulating antibodies, although the data obtained are suggestive of a correlation.

960

Blizzard, R.M.; Chandler, R.W.; Landing, B.H.; Pettit, M.D.; West, C.W. 1960. Maternal autoimmunization to thyroid as a probable cause of athyrotic cretinism. New Eng. J. Med. 263:327-336.

Sera of mothers of cretins have been tested for antithyroid antibodies by means of agglutinating, precipitating, complement-fixing, and Coons techniques. The frequency of occurrence of antithyroid antibodies in this group has been compared with that for the pregnant and general population. Antithyroid antibodies were found to occur significantly more frequently in the sera of mothers of cretins than in the other groups. Two sera of mothers of cretins were also found to contain a cytotoxic factor for thyroid tissue grown in tissue culture. The other sera were not tested for this factor. These data suggest that cretinism is sometimes causally related to thyroidal autoimmunization in the mother. It was demonstrated that when antithyroid antibodies cross the placenta, normal children or athyrotic cretins may be born, and that these antibodies disappear in the first four months of life. Although the presence of antithyroid antibodies in the serum of a pregnant woman only occasionally results in the birth of a cretin, the newborn infant of any woman who has a history of thyroid disease, and who has demonstrable circulating antithyroid antibodies, should be closely followed for signs and symptoms of hypothyroidism.

970

Cochrane, C.G.; Vazquez, J.J.; Dixon, F.J. 1957. The specific localization of antigen in lesions of experimental serum sickness. Amer. J. Pathol. 33:593-594.

Injections of bovine serum albumin, BSA, labeled with iodine 131, into rabbits produced lesions of proliferative glomerulitis, coronary arteritis, and endocarditis. Most lesions appeared before complete elimination of the BSA from circulation. Using FA, concentrations of BSA were found in the glomeruli, and BSA plus the gamma globulin of the host were found in arterial lesions. No BSA or concentrations of gamma globulin were seen before lesions developed. Controls indicated the reactions to be specific. It was concluded that antigen is specifically localized in the lesions of serum sickness at the time they are produced.

930

Cruickshank, B. 1959. The role of autoantibodies in anaphylactoid purpura. *Immunol.* 2:123-126.

Sera from cases of anaphylactoid purpura have been examined for precipitating, agglutinating, and complement-fixing antibodies against various extracts of normal human artery, kidney, and glomeruli and against cell-free arterial reticulin and glomerular basement membrane. The presence of tissue-localizing antibodies was investigated with the fluorescent antibody method. No conclusive evidence has been found for the presence of circulating tissue antibodies. Such reactions as were observed were nonspecific.

990

Dixon, F.J.; Feldman, J.D.; Vazquez, J.J. 1961. Experimental glomerulonephritis. *J. Exp. Med.* 113:899-920.

Daily injections of foreign proteins were used to produce various forms of renal disease in rabbits. The form of disease varied in correlation to antibody production. Fluorescent antibody was used to follow morphological aspects of the problem. This experimental model implied that this renal injury is precipitated by antigens with no known relationship to kidney, that antigen-antibody complexes localize in the kidney and may be etiologic in renal injury, that severe hypersensitivity disorders can be related to poor and good antibody responses, and that this pathogenesis suggests an alternative to the use of nephrotoxic serum for experiments in human glomerulonephritis.

1000

Dixon, F.J.; Vazquez, J.J.; Weigle, W.O.; Cochrane, C.G. 1958. Pathogenesis of serum sickness. *A.M.A. Arch. Pathol.* 65:18-28.

On the basis of the present observations it appears that the initiation and development of the morphologic manifestations of a serum sickness closely parallel the increase in rate of antibody formation. They occur while antigen-antibody complexes presumably capable of inducing inflammatory and anaphylactic responses are present in the circulation and are accompanied by an almost simultaneous localization of antigen and probably of antibody in the sites of the lesions. Also, there is no detectable localization or fixation of antigen in those tissues predisposed to the development of lesions prior to the formation of antibody.

1010

Doniach, D.; Hudson, R.V. 1961. Hashimoto disease with complement-fixing antibodies to several human organs. *Proc. Roy. Soc. Med.* 53:871-872.

In this case history of Hashimoto disease, FA revealed antinuclear factor when the LE cell test was negative.

1020

Doniach, D.; Roitt, I.M. 1961. Present concepts of thyroid autoimmunity, p. 278-298. In H. Gardiner-Hill, ed., Modern trends in endocrinology. Hoeber Medical Division, Harper and Row, Publishers, New York.

The fluorescent antibody technique is indicated among other tests for the detection of thyroid autoantibodies. This is a general discussion of aspects and potentials in the study of the thyroid.

1030

Fennell, R.H.; Rodnan, G.P.; Vazquez, J.J. 1962. Variability of tissue-localizing properties of serum from patients with different disease states. Lab. Invest. 11:24-31.

The sera from patients with systemic lupus erythematosus, scleroderma, rheumatoid arthritis, Hashimoto thyroiditis, malignant hypertension, and dermatomyositis were tested for the presence of nuclear-localizing antibodies by the fluorescent antibody technique. Three of 21 serum specimens from patients with rheumatoid arthritis and 2 of 12 serum specimens from patients with Hashimoto thyroiditis were found by this technique to contain nuclear-localizing factors identical to the factor found with lupus serum. The serum specimens from other patients showed variable or negative results. It is suggested that the presence of nuclear-localizing factors is not a basis for pathogenetic classification of disease, and the development of the factor or factors may be a secondary phenomenon.

1040

Freedman, P.; Markowitz, A.S. 1959. Immunological studies in nephritis. Lancet 2: 45-46.

A serum factor distinct from albumin, alpha-1, alpha-2, beta, and gamma globulins, and reacting immunologically as complement, was localized in the glomeruli in a small series of biopsy and fresh necropsy kidney tissue from patients with glomerulonephritis and lupus nephritis and from one patient with diabetic nephropathy who had not received insulin. Negative results were obtained in histologically normal kidney in a case of scleroderma with doubtful histological changes in the glomeruli and in a case of mild proliferative and membranous glomerulonephritis associated with Henoch-Schonlein purpura. In the absence of a pure preparation of human complement, however, interpretation of these results can be based on inference only.

1050

Freedman, P.; Peters, J.; Karl, R. 1960. Localization of gamma globulin in the diseased kidney. A.M.A. Arch. Intern. Med. 105:524-535.

Renal tissue from 43 patients has been studied by fluorescent antibody for gamma globulin. A high incidence of glomerular-localized globulin was noted in the kidneys of patients with glomerulonephritis at all stages and in the renal lesions associated with systemic lupus erythematosus, progressive systemic sclerosis, polyarteritis, amylohexosis, and diabetes mellitus. Specific fluorescence indicative of the presence of gamma globulin was

also seen in the walls of the small blood vessels in a smaller proportion of these patients. Elution procedures at pH 3.3 led to some loss of gamma globulin from the kidney tissue, as indicated by the fluorescent antibody stains and also by tests of the eluate for gamma globulin. These findings provide support for an immunological reaction in the pathogenesis of some forms of glomerulonephritis and the so-called connective tissue diseases.

1060

Hijmans, W.; Doniach, D.; Roitt, I.M.; Holborow, E.J. 1961. Serological overlap between lupus erythematosus, rheumatoid arthritis, and thyroid autoimmune disease. *Brit. Med. J.* 2:909-914.

Patients with the subject diseases were tested for autoantibody production. Antinuclear factor and thyroid antibody tests were done by FA. Results indicated that the autoimmune diseases form a broad overlapping spectrum with antigen disturbances at one end and antibody production disturbances at the other.

1070

Holborow, E.J.; Brown, P.C.; Roitt, I.M.; Doniach, D. 1959. Cytoplasmic localization of complement-fixing autoantigen in human thyroid epithelium. *Brit. J. Exp. Pathol.* 40: 583-588.

Sections of human thyroid were treated with chronic thyroiditis sera, and globulin was demonstrated by application of fluorescein-conjugated antihuman globulin serum. Thyroiditis sera containing thyroglobulin precipitins gave rise to specific staining of the follicular colloid in alcohol-fixed sections. Staining of the cytoplasm of acinar cells in unfixed sections was observed, using patients' sera capable of reacting with the complement-fixing thyroid microsomal antigen. Cytoplasmic staining was abolished by absorption with a thyroid microsome preparation or by pretreatment of the thyroid section with alcohol. This staining was unaffected by absorption of the serum with thyroglobulin or with a human liver microsome preparation. Staining was organ-specific. Strong cross-reactions were obtained with monkey thyroid, but rabbit thyroid gave only weak staining. The autoantigen present in the microsome fraction of thyroid homogenates is derived from the cytoplasm of the epithelial cells and is distinct from thyroglobulin.

1080

Irvine, W.J. 1960. The cytotoxic factor in thyroid disease. *Scot. Med. J.* 5:511-522.

The cytotoxic effect of sera from patients with certain thyroid diseases on hyperplastic human thyroid cells in tissue culture is described and illustrated. The thyroid cytotoxic factor has proved to be specific for thyroid tissue and is invariably present in untreated Hashimoto patients, in most patients with spontaneous hypothyroidism, and in more than 50 per cent of patients with thyrotoxicosis. Thyroid cells vary in their susceptibility to the cytotoxic factor; hyperplastic cells are the most susceptible and cells from a simple goiter are the least susceptible. It is likely that the cytotoxic effect is related to an antigen-antibody reaction occurring at the cell membrane in the presence of complement and is independent of the thyroglobulin and thyroid microsomal autoimmune systems.

1090

Konda, S.; Yamada, A.; Fukase, M.; Hamashima, Y.; Kawamura, M. 1961. The demonstration of incomplete antibody in autoimmune hemolytic anemia by the fluorescent antihuman globulin serum technique. *Acta Sch. Med. Univ. Kioto* 37:94-89.

Specific fluorescent membranes were demonstrated around erythrocytes in some cases of autoimmune hemolytic anemia using the antihuman globulin serum conjugated with fluorescein. This fluorescent membrane seemed equal in quality to that visualized around A erythrocyte agglutinated with heterogeneous specific anti-A serum. In three cases of autoimmune hemolytic anemia the fluorescent membranes were demonstrated before prednisolone treatment but extinguished after the treatment, despite their remaining positive in direct Coombs test. The mechanism of quenching is still obscure. Fluorescence could not be visualized around O erythrocytes agglutinated by pan-erythrocyte agglutinin.

1100

Lange, K.; Wachstein, M.; McPherson, S.E. 1961. Immunologic mechanism of transmission of experimental glomerulonephritis in parabiotic rats. *Proc. Soc. Exp. Biol. Med.* 106: 13-16.

The glomerular basement membranes in kidneys of rats made nephritic by antirat-kidney rabbit serum show the presence of rabbit gamma globulin when examined with a fluorescein-labeled antirabbit gamma globulin serum. Rat gamma globulin, presumably rat antibody, appears only after 6 days on these membranes. The parabiosis of such nephritic rats to healthy rats leads to a mild but clearcut nephritis in the healthy partner with proteinuria and typical histologic changes. Rat gamma globulin, presumably antibody, but not rabbit gamma globulin can be shown on the glomeruli of such secondarily diseased animals. The parabiosis of two normal rats does not lead to such deposition of antibody or to clinical disease. Thus the primary nephritic partner seems to form an autoantibody against its own altered kidney tissue. This antibody is then in turn able to attack the healthy kidney tissue of the secondary partner.

1110

Lange, K.; Wenk, E.J.; Wachstein, M.; Noble, J. 1958. The mechanism of experimental glomerulonephritis produced in rabbits by avian antikidney sera. *Amer. J. Med. Sci.* 236: 767-778.

Experimental glomerulonephritis was produced in rabbits by injection of duck or chicken antiserum to rabbit kidney following a 4- to 5-day delay period. The onset time was not modified by replacement of part of the duck antiserum with normal duck or human serum. The disease was not induced in rabbits by transfusion of nephritic or antiduck rabbit serum, whether or not the recipient rabbit had been sensitized. Therefore, the disease-producing antibody was specific for the localizing factor. Fluorescent antirabbit gamma globulin localized in the glomeruli when the disease appeared, not before. Fluorescent antiduck globulin and nephritic rabbit serum localized immediately following administration of duck serum, the former giving weak fluorescence and the latter giving intense fluorescence. After disease appeared, this staining did not occur; autoantibodies apparently occupied the antigen sites. Nephritis in mammals following injection of avian serum is therefore caused by antibody produced in the recipient animal and is a biphasic disease. Monophasic disease is caused by injection of mammalian antiserum.

1120

Lessef, M.H.; Crawford, H.J.; Wood, R.M. 1959. Antibody to thyroglobulin in patients with thyroid disease. *Lancet* 2:1172-1173.

A slide test employing spots of antigen made fluorescent by specific fluorescein-labeled antiglobulin is described. Its use in the study of thyroglobulin from human patients with various thyroid conditions is described, and a comparison is made with the passive cutaneous anaphylactic reaction in the guinea pig. The fluorescent-spot test appeared to be the more sensitive test.

1130

Macotela-Ruiz, E. 1962. The fluorescent antibody technique: Applications in experimental dermatology. *Frenza Med. Mex.* 27:163-172. In Spanish.

This is a review of methods and uses, directed principally toward dermatology and autoimmune diseases.

1140

Mancini, R.E.; Vilar, O.; Dellacha, J.M.; Davidson, O.W.; Castro, A. 1961. Histological localization in rat tissues of intravenously injected thyrotrophin labeled with a fluorescent dye. *J. Histochem. Cytochem.* 9:271-277.

Thyrotrophin preparations were conjugated with lissamine rhodamine RB 200. Biologic activity loss was 12 to 34 per cent. Rat groups were injected intravenously with labeled thyrotrophin, the same but completely denatured, and dye solution. Thyrotrophin remained in the circulation longer than the denatured material, but both declined. Thyrotrophin was found in the thyroid gland, ocular connective tissue, skeletal muscle interstitium, perivisceral adipose tissue, and mast cells. Kupffer cells and other macrophages and proximal tubule cells showed the most long-lasting accumulation. Denatured thyrotrophin was not in the thyroid, and in low quantity in connective and adipose tissue and in mast cells. More was present in macrophages and convoluted tubule cells, and it was eliminated through the small intestine epithelium. Plain dye was eliminated via the urinary and bile systems.

1150

Mellors, R.C. 1955. Pathogenesis of experimental glomerulonephritis: Histopathological demonstration of glomerular-localizing antibodies. *Amer. J. Pathol.* 31:592.

The role of antigen-antibody reactions in the pathogenesis of certain types of glomerulonephritis, as well as of a variety of other diseases thought to be associated with allergy and hypersensitivity, has been discussed widely during the past century. Clinical observations, serologic findings, morphologic studies, and investigative work with experimental animals lend indirect support to immuno-allergic pathogenesis. The unique feature of the present study is the use of recently described microfluorescence methods for demonstrating the histologic sites of localization of antibodies in vivo. Antibodies are localized in the glomeruli of the kidneys of rabbits having glomerulonephritis of

the acute proliferative, the exudative, or the crescentic type induced by the injection of foreign protein as bovine gamma globulins. Such localization is clearly a requisite for the allergic pathogenesis of glomerulonephritis. Complete article.

1160

Mellors, R.C. 1955. Histochemical demonstration of the *in vivo* localization of antibodies: Antigenic components of the kidney and the pathogenesis of glomerulonephritis. *J. Histochem. Cytochem.* 3:284-289.

The role of antigen-antibody reactions in the pathogenesis of certain types of glomerulonephritis and in the development of various lesions of the heart, blood vessels, skin, and joints in allergic, hypersensitive, and other pathologic states has been discussed widely during the past half-century. A method, the microfluorescence technique, for the histologic demonstration of the antigenic components of tissues and the sites of localization of antibodies should provide further knowledge concerning the role of antigen-antibody interactions in these diseases.

1170

Mellors, R.C.; Arias-Stella, J.; Siegel, M.; Pressman, D. 1955. Analytical pathology: II. Histopathologic demonstration of glomerular-localizing antibodies in experimental glomerulonephritis. *Amer. J. Pathol.* 31:687-715.

Of 15 rabbits receiving one or more intravenous injections of purified foreign proteins as bovine gamma globulins, 12 developed glomerulonephritis; 8, myocarditis; 3, endocarditis; 5, interstitial pneumonia; 4, allergic angiitis; 15, lymphoid hyperplasia of the spleen; and 2, allergic granulomas of the spleen. The evidence of renal injury in 12 animals consisted of proteinuria, renal enlargement, renal petechiae, tubular casts, and glomerulitis. Antibodies were localized in the glomeruli of the kidneys of rabbits having acute glomerulonephritis. Such localization, not heretofore demonstrable, is clearly a requisite for the allergic pathogenesis of glomerulonephritis. Studies of other organs in these sensitized animals suggested that antibodies were localized in the blood vessels in angiitis and in the heart in endocarditis and myocarditis. The analytical method used should permit the study of the role of antigen-antibody interactions in the pathogenesis of a number of human diseases.

1180

Mellors, R.C.; Arias-Stella, J.; Siegel, M.; Pressman, D. 1955. Demonstration of the role of glomerular-localizing antibodies in pathogenesis of experimental glomerulonephritis. *Federation. Proc.* 14:821-254.

A microfluorescence method for the histological demonstration of the sites of *in vivo* localization of antibodies has recently been developed. After treatment of tissue sections by an immunochemical fluorochroming procedure, sites of antibody localization become self-luminous when viewed with the fluorescence microscope. The role of antigen-antibody interactions in the pathogenesis of a number of experimental and clinical diseases was studied. Of 15 rabbits rendered hypersensitive by the intravenous injection of purified foreign protein as bovine gamma globulin, 12 developed glomerulonephritis; 8, myocarditis; 3, endocarditis; 5, interstitial pneumonia; and 4, allergic angiitis.

By the microfluorescence method, utilizing a quantitative photographic photometer and analyzing the results statistically in comparison with control data, antibody localization was clearly demonstrated in the glomeruli of the kidneys of rattits having glomerulonephritis of the proliferative, exudative, or crescentic type. Preliminary studies of other organs in the sensitized animals suggested that antibodies were localized in the blood vessels in angiitis and in the heart in endo- and myo-carditis. Complete article.

1190

Mellors, R.C.; Brzosko, W.J.; Sonkin, I.S. 1962. Immunopathology of chronic nonspecific thyroiditis or autoimmune thyroiditis. Amer. J. Pathol. 41:425-437.

Thyroglobulin, antibody to thyroglobulin, and mixtures of the two, apparently comprising immune complexes, were identified *in situ* in thyroid sections. Thyroglobulin, detected as antigen with fluorescent rabbit antibody, was distributed mainly in colloid within thyroid follicles but also outside of the follicles in close relation to mononuclear mesenchymal cells in the dense interstitial infiltrate. Cells containing and apparently forming antibody to thyroglobulin were abundant in the dense mononuclear cell infiltrate surrounding, invading, and replacing the follicular epithelium and the colloid. The majority of the antibody-containing cells were plasma cells. The antibody was 7S gamma globulin. Thyroglobulin with a globular appearance was intimately associated with 7S gamma globulin within and outside the thyroid follicles. Thyroglobulin and antibody to it were present in some degenerated epithelial cells. In addition, a few solitary cells containing globules of thyroglobulin and antibody were interpreted as being macrophages with phagocytized immune complexes. The major components of an autoimmune response - autoantigen, autoantibody, and apparently immune complexes - have all been demonstrated *in situ* in chronic nonspecific thyroiditis. This form of thyroiditis merits the designation autoimmune thyroiditis.

1200

Mellors, R.C.; Ortega, L.G. 1956. Analytical pathology: III. New observations on the pathogenesis of glomerulonephritis, lipid nephrosis, periarteritis nodosa, and secondary amyloidosis in man. Amer. J. Pathol. 32:455-499.

This study shows the histopathologic application of a microfluorescence method for demonstrating the histologic sites of localized human gamma globulins. It is established that gamma globulins are localized in the active glomerular lesions in lipid nephrosis of childhood and glomerulonephritis occurring in man at all ages. Gamma globulins are localized in the glomerular and the arterial lesions in periarteritis nodosa and in glomerular lesions in renal amyloidosis. By demonstrating that antibody proteins are localized in the sites of the lesions, the observations fulfill a requirement essential for the immuno-allergic pathogenesis of these several diseases of man. Localized gamma globulins are, at least in part, antibodies against antigens that have localized in the protective sites of the lesions. This investigation suggests that the pathogenesis of glomerulonephritis initiated in eclampsia of pregnancy, rheumatic fever, rheumatoid arthritis, disseminated lupus erythematosus, and other so-called collagen diseases will be further elucidated by the analytical procedure used in this study.

1210

Nairn, R.C.; Ghose, T.; Porteous, I.B.; Urquhart, J.A. 1962. A routine immunofluorescence method for detecting autoantibodies. *J. Clin. Pathol.* 15:594-595.

The principle of demonstrating autoantibodies to thyroid by immunofluorescence is simple: the sera of patients with suspected autoimmune thyroiditis are applied to sections of human thyroid and any interaction of thyroid antigen with specific autoantibody is detected by a fluorescent conjugate of antihuman globulin. Unfixed fresh frozen sections of thyroid are used to identify the complement-fixing anticytoplasmic autoantibody, but these are inadequate for identifying anticolloid activity because thyroid colloid is poorly retained in unfixed sections. To preserve colloid without denaturation in frozen sections, Balfour et al. employed a methanol fixation procedure that has usually given satisfactory results in our hands but suffers from certain disadvantages. In particular, stocks of thyroid tissue blocks, even when kept at -76°C, deteriorate rapidly and require replacement about every 3 weeks; fresh microscopical sections are required for every testing and there is always an uncertainty about the colloid fixation or about the suitability of a new thyroid gland that may also be difficult to obtain at the appropriate time. Balfour also used freeze-substitution of thyroid tissue but we find the method unsuitable for routine use: colloid seems often to be lost into the dehydrating solvent. These objections have been overcome by using lyophilized thyroid embedded in polyester wax and sectioned with a microtome. Such preparations provide an effective substrate for repeated autoantibody testing over many months.

1220

Ortega, L.G.; Mellors, R.C. 1956. Analytical pathology: IV. The role of localized antibodies in the pathogenesis of nephrotoxic nephritis in the rat. *J. Exp. Med.* 104: 151-170.

The principal objective of this investigation was to define the roles of injected and autogenous kidney-localizing antibodies in the pathogenesis of rat nephrotoxic nephritis by relating data obtained with fluorescent antibody techniques to clinical and histologic observations. Such an analysis of the nephritis that developed in 28 rats after injection with the crude gamma globulin fraction of nephrotoxic serum has led us to the following conclusions: The renal localization of nephrotoxic antibodies is primarily and perhaps exclusively in the membranes of the glomerular tufts. These antibodies are demonstrable, as antigens, in the glomeruli for as long as 3 months after injection. In the acute stage of the nephritis, nonantibody autogenous globulins are present in the glomerular tufts, probably as components of edema fluid. From 6 and 9 days to 3 months after injection, autogenous antibodies are localized in the glomerular tufts in a pattern that corresponds closely to that of the nephrotoxins.

1230

Porter, D.D.; Fennell, R.H. 1961. Serologic and immunohistochemical study of human and experimental thyroiditis. *New Eng. J. Med.* 265:830-834.

Material from 13 cases of Hashimoto thyroiditis was examined in an attempt to correlate serologic and morphologic findings. No correlation between the degree of thyroid destruction and the presence or titer of circulating antibodies or antithyroid could be found.

No single serologic test was consistently diagnostic of the condition. A reasonable explanation for antithyroid antibodies is that they represent a secondary manifestation of thyroiditis and not the pathogenic agent. Experimental thyroiditis was produced in three of 20 experimental rabbits. It did not closely resemble Hashimoto thyroiditis serologically or morphologically.

1240

Pulvertaft, R.J.V.; Doniach, D.; Roitt, I.M. 1961. The cytotoxic factor in Hashimoto disease and its incidence in other thyroid disease. *Brit. J. Exp. Pathol.* 42:496-503.

Trypsinized human thyroid cells in monolayer cultures are killed by Hashimoto sera in the presence of complement. If complement is inactivated by heating or absorbed out by an indifferent antigen-antibody complex, the cytotoxic effect is abolished. The factor is species- and organ-specific and consistently affects only cells from thyrotoxic and Hashimoto goiters. The factor is strongest in Hashimoto sera but is also present in low concentration in two-thirds of thyrotoxic sera and to a lesser extent in other thyroid diseases.

1250

Roitt, I.M.; Doniach, D. 1960. Thyroid autoimmunity. *Brit. Med. Bull.* 16:152-158.

Uses and implications of the FA test in the study of thyroid autoantigens are indicated in review. Other tests are also discussed in relation to the general thyroid autoimmunity problem.

1260

Roitt, I.M.; Doniach, D.; Couchman, K. 1960. Studies of an intracellular thyroid autoantigen, p. 70-78. In *Mechanisms of antibody formation*. Publishing House of the Czechoslovak Academy of Sciences, Prague.

A thyroid autoantigen exists. Hypotheses for this phenomenon are discussed.

1270

Seegal, B.C. 1962. The value of the fluorescent antibody technique in the study of chronic disease. *J. Chron. Dis.* 15:935-940.

This review article emphasizes the chronic diseases of various etiologies. Included are discussions on microorganism-related disease, connective tissue disorders such as rheumatoid arthritis and lupus erythematosus, endocrinological problems such as Hashimoto disease, nerve demyelination mechanisms, and tumor factors such as viruses and abnormal antigens. Studies of myasthenia gravis, kidney disease, and rheumatic fever are discussed. Both diagnostic applications and mechanism of pathogenesis studies are discussed.

1280

Seegal, B.C.; Hsu, K.C.; Rothenberg, M.S.; Chapeau, M.D. 1962. Studies of the mechanism of experimental nephritis with fluorescein-labeled antibody: II. Localization and persistence of injected rabbit or duck anti-rat-kidney serum during the course of nephritis in rats. Amer. J. Pathol. 41:183-204.

The course of acute, subacute, and chronic nephritis produced in the rat by the injection of rabbit or duck antiserum to rat kidney was followed for periods up to 291 days. Both injected rabbit and duck globulin were demonstrated in the glomeruli of nephritic animals. The rats that had the most severe acute nephritis showed the greatest amount of nephro-toxic globulin localized in the glomeruli. Progression of renal lesions to chronic glomerulonephritis occurred most rapidly in those animals that suffered the most severe acute disease. Cells in the spleen and adrenal contained small amounts of rabbit or duck globulin up to 5 months after injection. This was the case also in animals given injections of duck antiserum to rat lung. Rabbit or duck antiserum to rat kidney localized in rat glomeruli, as in the case of antisera to rat lung, initiated nephritis; progression of this might be attributable to persistence of the foreign globulin in the glomeruli.

1290

Stoloff, I.L. 1960. Fluorescence microscopy in diseases of the thyroid gland. J. Clin. Invest. 39:1033.

Cryostat sections of normal human thyroid were covered with the sera to be tested for 30 minutes, washed for 10 minutes with phosphate buffer at pH 7.3, covered with rabbit anti-human gamma globulin labeled with fluorescein isothiocyanate for 30 minutes, and washed in running tap water overnight. The sera of five normal persons and ten patients with a variety of diseases of the thyroid were tested. Brilliant fluorescence was seen in the acinar and interstitial cells of the sections treated with the sera of three patients with Hashimoto disease and one patient with a Riedel struma. Strikingly little fluorescence was seen on the colloid. Traces of fluorescence were seen in the sections treated with the sera of two patients with idiopathic hypothyroidism without goiter, two patients with euthyroid goiters, and two patients with adolescent goiter. The sections treated with the sera of the five normal persons were negative. Sections treated with sera of two patients with Hashimoto disease obtained 7 months after thyroidectomy were also negative, although a serum obtained from one of these preoperatively had been positive. Precipitins to thyroid extract were found in varying amounts in nine of ten sera of patients with thyroid disease and in none of five controls.

1300

Teodoru, C.V.; Brancato, P.; Volk, B.W. 1962. The connective tissue as systemic site for binding of duck anti-rabbit-kidney nephrotoxic serum. Federation Proc. 21:167.

The intra-articular administration of duck anti-rabbit-kidney serum, DARKS, to rabbits causes severe delayed arthritis and its intradermal injection produces local skin necrosis. To explain this apparent lack of specificity we attempted to investigate the sites of the immuno-allergic reactions and thereby find the common morphologic denominator for these diversified organic alterations induced by DARKS. Sections of normal kidney, liver, muscle, joint capsule, etc. were incubated with fluorescein-labeled DARKS gamma globulins.

There was selective fluorescence of interstitial and perivascular connective tissue. Control sections similarly treated with fluorescent normal duck serum gamma globulins failed to stain. In rabbits that received DARKS intravenously and were sacrificed 7 days later, kidney sections incubated with fluorescent antibodies against DARKS gamma globulins showed selective fluorescence of the interstitial connective tissue and the basement membrane of the glomerular tufts. Similarly, when fluorescent anti-DARKS gamma globulins were incubated with sections of periarticular tissue from rabbits that had received DARKS intra-articularly 3 months previously, the connective tissue was also selectively stained. These findings strongly suggest an antigen-antibody relationship between DARKS gamma globulins and rabbit connective tissue. This could account for the variety of delayed organic lesions provoked by DARKS, depending on the route of administration.

1310

Triedman, R.S.; Metzger, H.; Hsu, K.C.; Rothenburg, M.S.; Seegal, B.C.; Urquhart, J.A. 1962. Studies of the mechanism of experimental nephritis with fluorescein-labeled antibody: I. Localization and persistence of injected duck anti-rat-lung serum during the course of nephritis in rats. Amer. J. Pathol. 41:95-117.

Duck antiserum to rat lung produced nephritis in rats similar in course and histologic features to that which has followed the injection of other nephrotic antisera. The nephrotoxic duck globulin localized primarily in the glomeruli and persisted there during the entire period of observation up to 259 days. The amount of duck antiglobulin to rat lung bound in the glomeruli was relatively greater when the initial nephritis was severe but diminished gradually with time to about 10 per cent after 8 months. Duck globulin was found in the lungs of only 3 rats injected with duck antiserum to rat lung. Cells of the reticuloendothelial system, particularly those in the spleen and adrenal, contained small amounts of duck globulin up to 5 months after injection. Localization of duck antiserum to rat lung in glomeruli of the rat initiates nephritis, and progression of the disease may result from persistence of foreign globulin in the glomeruli.

1320

White, R.G. 1957. Discussion on autoimmunity and thyroiditis. Proc. Roy. Soc. Med. 50: 953-954.

In the study of Hashimoto disease lymph nodes were examined by FA. Specific antibody was demonstrated in reticuloendothelial cells located in the peripheral lymph sinuses of the nodes.

1330

White, R.G.; Marshall, A.H.E. 1962. The autoimmune response in myasthenia gravis. Lancet 2:120-123.

A series of cases is presented. FA was used to demonstrate antibodies against nuclei. A relationship between myasthenia and systemic lupus erythematosus is suggested.

1340

Wood, C.; White, R.G. 1956. Experimental glomerulonephritis produced in mice by subcutaneous injections of heat-killed Proteus mirabilis. Brit. J. Exp. Pathol. 37:49-59.

Acute or subacute diffuse glomerulonephritis was produced in about 50 per cent of mice by repeated subcutaneous injections of heat-killed P. mirabilis continued for 2 to 11 weeks. Using fluorescein-labeled antibody, antigenic material from P. mirabilis was found to be localized in the glomerular cells. After a single subcutaneous dose, antigen persisted for at least 3 weeks in glomeruli. It is suggested that the localization of bacterial antigen in glomerular cells may be causally related to the production of nephritis and that this localization site is related to the type of glomerulonephritis produced in mice, which resembled Ellis type 1 nephritis in man.

III. BLOOD: CELLULAR COMPONENTS

A. ERYTHROCYTES

1350

Clayton, R.M. 1959. Changes in the antigenic constitution of members of groups of cells. *Exp. Cell Res. Suppl.* 7:275-278.

A method of detecting tissue cells with homozygous reactions for blood group substances in the tissues of heterozygous individuals is described. Such cells tend to occur in clusters. The method may serve to compare the numbers of modified cells in different tissues of the same individual.

1360

Cohen, F.; Zuelzer, W.W.; Evans, M. 1960. Identification of blood group antigens and minor cell populations by the fluorescent antibody method. *Blood* 15:884-900.

It is possible to produce fluorescence of erythrocytes as the result of specific antigen-antibody reactions of various blood group antigens. Factors A and B, a variety of Rh antigens and Kidd, have been demonstrated with this method. The method discriminates between A1 and A2 cells. More antibody must be attached to the red cell for fluorescence than for agglutination. The relative paucity of antigenic sites of Rh substance compared with A and B antigens is reflected by the difference in intensity of staining. The fluorescent antibody technique has been used successfully for the demonstration and quantitation of minor cell populations in both *in vitro* mixtures and *in vivo*. Injected Rh-positive erythrocytes were demonstrated in the blood of an Rh-negative volunteer during a period of twenty days. Fetal Rh-positive erythrocytes were demonstrated in several Rh-negative women, both with and without antibody, in the last trimester of gestation.

1370

Cohen, F.; Zuelzer, W.W.; Evans, M. 1960. Identification of erythrocyte blood group antigens, and the demonstration of minor cell populations with fluorescent antibodies. *Federation Proc.* 19:69.

Demonstration of blood group antigens in erythrocytes by specific fluorescence was achieved by treating wet preparations of red cells with fluorescein isothiocyanate-labeled antibodies. Group A and Group B antigens were demonstrated by the direct method using coupled human anti-A and anti-B sera. A variety of Rh antigens and Kidd were demonstrated by the indirect method, in which red cells were sensitized with their specific incomplete antibody and then treated with labeled rabbit antihuman globulin. Low intensity of fluorescence with these weaker antigens was nevertheless specific because it could be shown that under the conditions employed, green fluorescence was an all-or-none reaction and controls showed no fluorescence. The method has been used to identify and quantitate minor cell populations in artificial mixtures; e.g., group A cells in group O cells, Rh-positive cells in Rh-negative cells, in ratios up to 1:100,000. Injected Rh-positive erythrocytes were demonstrated in the blood of an Rh-negative volunteer for as long as three weeks. Fetal Rh-positive red cells were demonstrated in several Rh-negative women with and without antibody, in the last trimester of pregnancy. The method is being used to study mechanisms of isoimmunization.

1380

Glynn, L.E.; Holborow, E.J.; Johnson, G.D. 1957. The distribution of blood-group substances in human gastric and duodenal mucosa. Lancet 2:1083-1088.

Conjugates of fluorescein isocyanate with anti-A, anti-Le a, and anti-H sera have been used to identify blood-group substances in gastric and duodenal mucosa obtained from group A, group AB, and group O persons undergoing partial gastrectomy. The patterns of distribution of A, Le a, and H substance in different parts of the mucosa and the difference between secretors and nonsecretors are described.

1390

Hasebe, H. 1962. Identification of blood group antigens A, B, and D RHO on human erythrocytes by the fluorescent antibody technique. Jap. J. Legal Med. 16:311-324. In Japanese.

FA was used to detect blood group antigens in tissue, cells, blood stains, and human secretions. Erythrocytes could be specifically stained by a tube method or smear. Results were best by a three-step technique described. Higher-titered sera gave superior results. Optimum temperatures and reaction times were determined. Saline was the best suspending medium for A and B antigens, and 10 per cent bovine serum albumin was best for the D antigen by both stain methods and best for smears for A and B antigens. Absorption of sera with erythrocytes was the best way to reduce nonspecific fluorescence.

1400

Hasebe, H. 1962. Determination of ABC blood groups in human blood stains by the fluorescent antibody technique. Jap. J. Legal Med. 16:325-329.

Specific FA staining was seen when applied to dried blood crusts.

1410

Holborow, E.J.; Brown, P.C.; Glynn, L.E.; Hawes, M.D.; Gresham, G.A.; O'Brien, T.F.; Coombs, R.R.A. 1960. The distribution of the blood group A antigen in human tissues. Brit. J. Exp. Pathol. 41:430-437.

The distribution of the blood group A antigen in a variety of human tissues has been studied by two methods - the mixed agglutination method, using human immune anti-A serum, and the Coons fluorescence method using a conjugate of rabbit antiserum to human A substance. Good agreement in results was found between the methods, which show that the A antigen is widely distributed in human tissues, principally in epithelial tissues and in vascular endothelium.

1420

Jankovic, B.D. 1959. Specific staining of red cell antigens by the use of fluorescein-labelled antibody. *Acta Haematol.* 22:278-285.

Procedures showing that A, B, AB, and Rh-positive red cells can be specifically stained with fluorescent antibody are described. Evidence was found for the importance of a quantitative relationship between antigen and antibody involved in the reaction.

1430

Jankovic, B.D.; Arsenijevic, K. 1959. Histochemical demonstration of A and B antigens in platelets. *Nature* 183:695-696.

The authors reported the methods and results in specific staining of unfixed platelets using fluorescent antibody. The fluorescent antibody technique would be successful in immunohaematology.

1440

Kaplan, M.H. 1957. Immunofluorescent reactions of human sera with iso-antigens in human heart tissue: AB blood group substances and cardiolipin: Evidence of a thermolabile antibody to cardiolipin associated with inflammation. *Federation Proc.* 16:1796:419.

Blood group iso-antigens A and B were localized in capillary walls, endothelium, adventitia of blood vessels, and the connective tissue stroma. Specificity was confirmed by absorption of reactive sera with heart tissue homogenates or packed red cells of the homologous blood group. A second antigen in heart tissue was reactive with Wassermann-positive sera. This staining reaction was localized mainly within the sarcoplasm of the myocardial cell, particularly between the myofibrils and adjacent to the cell membrane, with only traces of staining present in the connective tissue stroma and blood vessels. Absorption of such sera with beef cardiolipin reduced staining, and absorption with human heart tissue homogenates prevented staining. This sarcoplasmic pattern of staining was observed also with acute-phase sera from patients with rheumatic fever, rheumatoid arthritis, and subacute bacterial endocarditis. In such sera, a thermolabile complementfixing antibody for beef cardiolipin was found and could be quantitated after decomplementing the sera with an immune precipitate. The titer correlated with the sedimentation rate and C-reactive protein and may reflect acute inflammation.

1450

Konda, S.; Yamada, A.; Fukase, M.; Hamashima, Y.; Kawamura, M. 1961. The demonstration of incomplete antibody in autoimmune hemolytic anemia by the fluorescent antihuman globulin serum technique. *Acta Scn. Med. Univ. Kioto* 37:84-89.

Specific fluorescent membranes were demonstrated around erythrocytes in some cases of autoimmune hemolytic anemia using the antihuman globulin serum conjugated with fluorescein. This fluorescent membrane seemed equal in quality to that visualized around A erythrocyte agglutinated with heterogeneous specific anti-A serum. In three cases of autoimmune hemolytic anemia the fluorescent membranes were demonstrated before prednisolone treatment but extinguished after the treatment, despite their remaining positive

in direct Ccombs test. The mechanism of quenching is still obscure. Fluorescence could not be visualized around C erythrocytes agglutinated by pan-erythrocyte agglutinin.

1460

Lee, R.E.; Vazquez, J.J. 1962. Immunocytochemical evidence for transplacental passage of erythrocytes. Lab. Invest. 11:580-584.

Evidence is presented for the transplacental passage of human erythrocytes from the mother to the baby and vice versa. Minor cell populations were detected by use of the fluorescent antibody technique in eight newborns and two mothers. The significance of these findings is discussed as they relate to immunologic tolerance.

1470

Marquez, M.H.; Sotelo, S.J. 1961. Fluorescent antibodies of the human blood groups. Prensa Med. Mex. 26:303-304.

Using FA, it was possible to demonstrate the site of action of the natural antibodies of the human blood groups on red cells. The site of action is the surface of red cells that are homologous with the antibodies in autoimmune anemias.

1480

Reed, T.E. 1961. Variable specificity of an anti-A serum labelled in three different ways. Acta Haematol. 25:355-360.

An anti-A serum from an immunized group B male was labelled, separately, with tritium, iodine 131, and fluorescein. Agglutination specificity for A red blood cells was retained in each case. Autoradiography with the tritiated anti-A and the iodinated anti-A failed to distinguish between A1 and O cells, however. Counting the gamma emission of the iodinated anti-A indicated a relative uptake of antiserum of 2.3:1.6:1 for A1, B, and O cells for one set of experimental conditions. These results suggest that non-antibody protein adsorbs onto red cells. Fluorescein labelling of whole cells, in contrast, was specific: A cells fluoresced brightly and B and O cells remained dark. A variable proportion of B or O stromata, however, fluoresced after incubation with the fluorescein-conjugated anti-A. It is not known why fluorescein labelling is more specific than tritium or iodine labelling or why stromata label nonspecifically with fluorescein but whole cells do not.

1485

Shahani, S.; Southam, A.L. 1962. Immunofluorescent study of the ABO blood group antigens in human spermatozoa. Amer. J. Obstet. Gynecol. 84:660-666.

The presence of blood group antigens A or B on human spermatozoa was demonstrated with FA. Spermatozoa carry a single ABO blood group antigen on their surfaces.

1490

Szulman, A.E. 1959. Histochemical distribution of blood group antigens A and B in human tissues. Federation Proc. 18:2008:509.

Human blood group substances A and B have been mapped in homologous human tissues by appropriate fluorescein-labelled human hyperimmune antisera applied to frozen sections of surgical and autopsy material. The specific antigens were found in three general locations: in the cytoplasm and secretions of cells of mucous glands, in the cell walls of vascular endothelium, and in stratified epithelial cells. Mucus-secreting cells containing antigen were found in the salivary and esophageal glands, in the gastric, duodenal, and enteric mucosa excluding the colon, in the Brunner glands, and in the mucus-secreting glands of the trachea, bronchi, and uterine cervix. In the pancreas the acinar cells contained large amounts of antigen in their cytoplasm, as did the secretions in the lumina of acini and ducts. The stratified epithelia showed good outlining of cell walls in the malpighian layer, well illustrated in the skin and esophagus. Transitional epithelium of the urinary tract, extending up into the cuboidal epithelium of the renal collecting tubules, also contained antigen. The vascular system was remarkable for the constancy of its content of antigen in the cell walls of the endothelium. Specificity was established by controls.

1500

Szulman, A.E. 1960. The histological distribution of blood group substances A and B in man. J. Exp. Med. 111:785-799.

FA was used to map histologically blood group antigens A and B in human tissues. A, B, and AB subject tissues were studied. Group O tissues were negative controls. Subject secretor status was determined. Secretor or nonsecretor status governs antigens associated with mucous secretions in most locations. Widespread antigen was demonstrated in the malpighian layers, transitional epithelium, and the basal and contiguous layers. Localization was also in the cell walls of endothelium of capillaries, veins, arteries, and sinusoidal cells of spleen.

1510

Szulman, A.E. 1962. The histological distribution of the blood group substances in man as disclosed by immunofluorescence: II. The H antigen and its relation to A and B antigens. J. Exp. Med. 115:977-996.

The H antigen was mapped out by immunofluorescence in human tissues from individuals of the various groups with the ABO system, both secretors and nonsecretors. Distribution of the antigen can be summarized: Cell walls of endothelium in the cardiovascular system; cell walls of stratified epithelia; skin, non-cornifying squamous stratified membranes, and transitional epithelia; mucus produced in secretor individuals and a few special areas in nonsecretors. In secretions and excretions, the antigen is distributed in the pancreatic and sudoral areas independent of secretor status, and in mammary and uterine tissues, as governed by the secretor makeup. The distribution of the H antigen is most fully represented in tissues of group O. It follows an over-all universal pattern, characteristically modified in nonsecretors. Within this pattern, in tissues of

the non-O-groups, the complement of the H substance in its various forms wanes in a manner consistent with the hypothesis that it serves as a substrate for the A1, A2, B genes, exerting their action with different degrees of efficiency.

1520

Wartman, W.B.; Tenczar, F.J. 1961. Specific staining of red cell antigens by use of fluorescent antibody, p. 362-363. In The year book of pathology and clinical pathology, 1960-1961 Series. Year Book Medical Pub., Chicago.

This article describes the use of FA for staining red blood cell antigens.

1530

Whitaker, J.; Zuelzer, W.W.; Robinson, A.R.; Evans, M. 1959. The use of the fluorescent antibody technique for the demonstration of erythrocyte antigens. J. Lab. Clin. Med. 54:282-283.

It has been shown that pretreatment of the red blood cells with chelating agents and agitation during the staining process intensifies the specific fluorescence of the erythrocyte membrane. Versene diminishes or abolishes the Coombs test on sensitized cells but has no effect on unsensitized cells.

1540

Yamasawa, K. 1962. Blood grouping of saliva and semen stains by the use of fluorescein-labelled antibodies. Jap. J. Legal Med. 16:238-241.

ABO blood groups may be identified in these specimens with FA. Simplicity may make this selective electrophoretic elimination of unbound antibodies widely applied in medico-legal examinations.

B. LEUKOCYTES

1550

Bedarida, G. 1962. Use of fluorescent antiglobulins in research on antileukocyte antibodies. Riv. Emoterap. Immunoematol. 9:2:95-111. In Italian.

This is a methods review. Nonspecific fluorescence reduction is discussed.

1560

Cochrane, C.G.; Weigle, W.O.; Dixon, F.J. 1959. Localization of antigen in the Arthus vasculitis and its removal by leukocytes. Federation Proc. 18:2206:562.

In Arthus reactions, the antigen rapidly localizes in the walls of affected blood vessels, in part, within leukocytes. Arthus reactions were induced with bovine albumin, BSA, in both control and rabbits treated with nitrogen mustard, HN2, to eliminate circulating polymorphs. In the absence of polymorphs, the BSA antigen was deposited within the walls of small vessels, usually beneath the intima. The BSA persisted for 48 hours in 11 of 13 cases; in control rabbits antigen persisted in the polymorph-infiltrated vessels in only two of ten rabbits. Persistence of antigen in polymorph-depleted rabbits was not due to the direct action of HN2 on the vessels. Leukocytes removed from Arthus reactions were found to contain sharp granular cytoplasmic fluorescence representing both BSA antigen and rabbit globulin. This was noted in many but not all polymorphs and in occasional mononuclear cells. Normal leukocytes did not contain these granules, and other fluorescent antibody solutions failed to show the granular fluorescence. These studies indicate that antigen and antibody react within vessel walls in the Arthus vasculitis and suggest that leukocytes, especially polymorphs, are instrumental in the removal of antigen.

1570

Cochrane, C.G.; Weigle, W.O.; Dixon, F.J. 1959. The role of polymorphonuclear leukocytes in the initiation and cessation of the Arthus vasculitis. J. Exp. Med. 110:481-494.

The role of polymorphs in the Arthus-type hypersensitivity vasculitis has been studied. Polymorphs were found to play an essential role not only in producing the inflammatory vasculitis, but also as instrumental in ridding the damaged vessel of the antigen, probably by means of proteolytic catabolism at the inflammatory site. A temporal relationship between the disappearance of antigen from the damaged vessels and a decrease in inflammatory reaction was found. The earliest localization of antigen and its associated rabbit globulin in the Arthus vasculitis was found beneath the endothelium of small vessels. Sorkin and Boyden reported the catabolism of antigen in the presence of antibody by mononuclear cells obtained from the peritoneal cavities of guinea pigs. The antigen molecule was actually broken down by the mononuclear cells.

1580

Daems, W.Th.; Oort, J. 1962. Electron microscopic and histochemical observations on polymorphonuclear leukocytes in the reversed Arthus reaction. *Exp. Cell Res.* 28:11-20.

Granulocytes in the reversed Arthus reaction provoked with a bovine albumin-antibovine albumin or a ferritin-antiferritin system were studied with the electron microscope and by means of enzyme histochemistry. The cells showed large intracytoplasmic electron-dense lumps, consisting of antigen-antibody precipitate, as could be demonstrated in the ferritin-antiferritin system. Furthermore, granules and mitochondria disappear from the cytoplasm, and the cells often show a vacuole situated underneath the nuclear membrane. These changes are discussed in relation to the findings in the literature. Part of the reaction is attributed to direct antigen-antibody interaction.

1590

Danaher, T.H.; Friou, G.J.; Finch, S.C. 1957. Fluorescein-labeled antiglobulin test applied to leukocyte immunology. *Clin. Res.* 5:9.

FA was used to study mechanisms of leukocyte antibody reactions. Fluorescein isocyanate-labelled antihuman globulin rabbit serum and antirabbit globulin chicken serum were prepared and absorbed with normal human erythrocytes. Staining and observation of normal human leukocyte smears indicated that heterologous antibody localized principally in leukocyte cytoplasm. Upon repeated absorption of labelled serum with leukocytes, titer comparisons of FA staining, Coombs titer, and globulin precipitation were made. Titer losses correlated. Other mammalian leukocytes fluoresced with antihuman rabbit conjugate only after prior incubation with normal human serum. Human globulin is present in or on normal human leukocytes, and human globulin firmly adheres to many types of heterologous leukocytes.

1600

Duheille, J.; Herbeauval, H.; Bellut, F.; Badonnel, Y. 1962. Immunofluorescent demonstration of the factors of anti-leukocyte sera. *Compt. Rend. Soc. Biol.* 156:2093-2099.

The application of direct and indirect FA to the immunology of leukocytes is described. These methods are particularly useful for lupus erythematosus.

1610

Herbeauval, R.; Herbeauval, H.; Duheille, J. 1962. Fluorescence and immunofluorescence in blood cytology. *Strasbourg Med.* 13:621-626. In French.

Applications of fluorescence microscopy and FA in the study of white cell concentrations for evidence of cancer are discussed. A review of the results of other workers and those of the authors is presented.

1620

Jankovic, B.D.; Arsenijevic, K. 1959. The demonstration of anti-leukocyte antibodies in human and rabbit immune serum by means of fluorescent antibody technique. *Acta Haematol.* 21:387-393.

Investigations of human and rabbit immune sera for the presence of anti-leukocyte antibodies by means of indirect fluorescent antibody have shown that it may be usefully applied in immunohaematology.

1630

Jankovic, B.D.; Lincoln, T.L. 1959. The demonstration of Rh antigen in human leukocytes. *Experientia* 15:61.

D antigen may be detected in white cells from Rh-positive persons by means of the fluorescent antibody technique. The staining technique consists of three steps: Incubation of leukocytes with anti-D serum, exposure of sensitized white cells to chicken antihuman globulin serum, and staining with antichicken globulin fluorescein conjugate. The leukocytes from Rh-positive and Rh-negative persons were investigated. The controls were washed cells, examined for autofluorescence; leukocytes directly exposed to fluorescein conjugate; sensitized white cells stained with fluorescein conjugate; and leukocytes treated with chicken antihuman globulin serum and incubated in normal human serum that did not contain anti-D antibody. A Reichert fluorescent microscope equipped with a high-pressure mercury vapor lamp and darkfield condenser was used. The photographs were taken on Agfa Fluorapid film that is particularly sensitive to yellow-green light. Positive results are obtained only with leukocytes from Rh-positive persons.

1640

Jankovic, B.D.; Lincoln, T.L. 1959. The presence of Rh antigen in human leukocytes as demonstrated by the fluorescent antibody technique. *Vox. Sang.* 4:119-126.

Using a three-step indirect fluorescent antibody technique, normal and myeloid leukocytes from Rh-positive persons were found to contain D antigen.

C. PLATELETS AND MEGAKARYOCYTES

1650

Duheille, J.; Herbeuval, R.; Herbeuval, H. 1962. Diagnosis of megakaryoblasts by immunofluorescence. Nouvelle Rev. Franc. Hematol. 2:621-625. In French.

Antigenic elements of megakaryocytes and platelets were identified. The advantages of the method are specific identification of abnormal blood elements and avoidance of diagnostic errors.

1660

Herbeuval, H.; Duheille, J. 1961. Immunofluorescent identification of megakaryocytes circulating in the blood. Compt. Rend. Soc. Biol. 155:2166-2170. In French.

Through antiplatelet immunofluorescent staining of the blood elements obtained from cell concentration, it was possible to specifically identify megakaryocytes. Confusion with other elements was avoided.

1670

Humphrey, J.H. 1955. Origin of blood platelets. Nature 176:38.

Apart from polymorphonuclear leukocytes, which had a bluish-grey fluorescence, the only cells in the smears that fluoresced were megakaryocytes, and these had the apple-green color of the fluorescein-coupled proteins. In smears treated with normal rabbit globulin conjugated with fluorescein, the polymorphonuclear leukocytes appeared the same, but megakaryocytes were unstained. Megakaryocytes must therefore share with platelets some characteristic unique antigenic structure, and this fact may be regarded as direct evidence of their relationship.

1680

Silber, R.; Benitez, P.; Eveland, W.C.; Akeroyd, J.H.; Dunne, C.J. 1960. The application of fluorescent antibody methods to the study of platelets. Blood 16:958-957.

Through the use of FA, it is possible to detect the antigen-antibody reaction between platelets and heterospecific antiserum, but no antigen-antibody reaction between normal human platelets and sera from patients with idiopathic thrombocytopenic purpura could be demonstrated. The method permits the histologic localization of platelets in splenic tissue. Although serum proteins can be easily removed from platelets by washing, there is an intimate association with fibrinogen, so that this protein is still not removed from platelets after 10 washings in saline.

1690

Sokal, G. 1962. Studies of the morphology of blood platelets and their viscous metamorphosis using fluorescent antifibrinogen and antiplatelet antiserum. *Acta Haematol.* Basel 28:313-325.

The morphology of platelets and their viscous metamorphosis were studied with two different fluorescein-conjugated antisera. One antiserum was specific for human fibrinogen, the second was against platelets from a case of congenital afibrinogenemia. The second, when tested in immunolectrophoresis, failed to react with fibrinogen. With platelet extract it developed a single precipitation line of beta 2 motility, which could not be detected in plasma. Both antisera, when tested on bone marrow smears, showed specific affinity for the cytoplasm of megakaryocytes. Fibrinogen could also be demonstrated to occur inside platelets, where it could be shown to condense within the center of those elements and their agglutinates during the process of viscous metamorphosis. The fibrinogen-free hyalomer was shown to be responsible for the formation of pseudopoda that were gradually resorbed after the platelet agglutinates had become incorporated into the fibrin meshwork. Platelet fibrinogen thus appeared to serve as a lately formed but lasting cement linking the platelet agglutinates and providing supporting centers for the plasma fibrin fibers.

1700

Sprague, C.C. 1961. The use of the fluorescent antibody technique in the detection of platelet antibodies, p. 689-697. In S.A. Johnson, R.W. Monto, J.W. Rebuck, and R.C. Horn, ed., *Blood platelets*. Little Brown, and Co., Boston.

Antisera to rat globulin and human platelets were prepared in rabbits and conjugated with fluorescein isothiocyanate. Specific staining of platelets and megakaryocytes was observed. Absorption experiments established common antigens between megakaryocytes and platelets. The direct FA method was preferred

1710

Vazquez, J.J.; Lewis, J.H. 1960. Immunocytochemical studies on platelets: The demonstration of a common antigen in human platelets and megakaryocytes. *Blood* 16:968-974.

By means of fluorescent antibody it was possible to demonstrate a common antigenic structure in human platelets and megakaryocytes, both in nonthrombocytopenic cases and in cases with idiopathic thrombocytopenic purpura. Direct evidence for a marked increase in the number of platelets in spleens of two cases of idiopathic thrombocytopenia is given. The pathogenic significance of this finding is discussed. The fluorescent antibody technique is a valuable tool for chemical and morphologic study of platelets and megakaryocytes in both tissues and smears.

IV. BLOOD: PLASMA PROTEINS

A. ORIGIN AND DISTRIBUTION

1720

Askonas, B.A.; White, R.G. 1956. Sites of antibody production in the guinea pig: The relation between in vitro syntheses of anti-ovalbumin and gamma globulin and distribution of antibody-containing plasma cells. Brit. J. Exp. Pathol. 37:61-74.

Foot-pad injections in guinea pigs using ovalbumin in Fruend adjuvant were made. Subsequently, tissues were examined for antibody-containing plasma cells and incorporation of Cl^{14} -glycine into gamma globulin in vitro. The ca-site granuloma and popliteal lymph node were relatively inactive for in vitro anti-ovalbumin production. Remote lymph nodes were highly active in the incorporation of Cl^{14} -glycine into the anti-ovalbumin, highest activity being in the contralateral flank. Of other organs examined, only spleen and bone marrow were also active. A good correlation was found between numbers of antibody-containing plasma cells and tissue ability to form anti-ovalbumin in vitro.

1730

Baney, R.N.; Vazquez, J.J.; Dixon, F.J. 1962. Cellular proliferation in relation to antibody synthesis. Proc. Soc. Exp. Biol. Med. 109:1-4.

A technical approach for the study of the kinetics of antibody-producing cells is possible by combination of autoradiography and the fluorescent antibody technique. Results show that: Almost all antibody-containing cells present by day 4 of a secondary response are newly formed cells arising from mitotic division of precursor sometime after antigenic stimulation; most plasma cells do not arise by direct, non-mitotic differentiation from lymphocytes; and functionally differentiated antibody-containing cells can divide.

1740

Baraugh, B.D. 1957. Site of production of antibodies. Indian J. Med. Sci. 11:917-936.

This is a review of literature on antibody production sites. The author briefly mentions the use of fluorescein-labeled antibody as a histochemical reagent in determining the source of antibody.

1750

Bardawil, W.A.; Toy, B.L.; Hertig, A.T. 1958. Localization of homologous plasma proteins in the human placenta by fluorescent antibody. Amer. J. Obstet. Gynecol. 73:708-717.

Normal and abnormal human placentas, as well as specimens of hydatidiform mole and choriocarcinoma, have been surveyed for homologous plasma albumin and globulin by the fluorescent antibody technique. Protein has been found broadly dispersed throughout the stromal ground substance of the villi, decidua, and cord, as well as in the intervillous spaces. Slight specific localization has been noted in the trophoblastic cytoplasm, but not within nuclei. Syncytial knots have shown somewhat higher levels of cytoplasmic

protein accumulation. Albumin and gamma globulin exhibited similar distribution, and all placental material, including that from a case of erythroblastosis fetalis, presented similar topography. Syncytial masses in the choriocarcinoma revealed intracytoplasmic localization, but the mole showed a general diffusion through the vesicular stroma. The findings do not indicate synthesis of blood protein by the human placenta, but rather point to a transfer of maternal albumin and globulin across the syncytium, perhaps by pinocytosis and subsequent discharge into the vilous stroma.

1760

Barnes, F.W., Jr.; Edgerton, M.T., Jr. 1960. Deposition of host serum proteins in homografts. Bull. Johns Hopkins Hosp. 106:250-251.

In this study fluorescent antibody was used to study frozen sections of homografts and auto- or iso-transplants. Collagen bundles of the dermis and some epidermal cells stained specifically in the homografts but not the other transplants. It was concluded that the constituents of the host serum invaded the homograft by 48 hours in a specific reaction.

1770

Barnes, F.W., Jr.; Seip, W.F.; Burch, C.C., Jr. 1962. Deposition of gamma globulin in homografts. Federation Proc. 21:38.

Earlier work showed that host serum protein accumulates in the skin homograft of the guinea pig, beginning prior to the 48th hour. Application of fluorescent techniques to isolated gamma globulin of rabbit antisera, obtained by injection of highly purified guinea pig serum albumin and gamma globulin, has now shown deposition of host gamma globulin in 2-day, 5-day, and second-set skin homografts in the guinea pig. This finding is not given by normal skin, autograft, or isograft. The gamma globulin is present in epidermis and in collagen bundles of dermis. The amount of deposition of gamma globulin was estimated. Factors explored in determining specificity of the antisera and variables in accurate use of fluorescent technique are discussed. The results are those predicted by a new recently announced theory of antibody formation and certain additions to the theory are made through the work reported here. This work was supported by a grant from the National Institutes of Health.

1780

Burkl, W.; Schwab, F. 1959. Histological and immunohistological demonstration of local antibody production in the cornea. Arch. Ophthalmol. 161:168-184.

Results of earlier serological studies suggested that intracorneal application of antigen leads to direct production of antibodies in the cornea. It may be demonstrated histologically that the influence of antigen elicits numerous plasma cells in the cornea, in addition to inflammatory irritation forms of corneal cells. The plasma cells contain the antigen, as demonstrated immunohistologically by the layering method. Whether or not a noncellular antibody distribution exists, one associated with the basic substance could not be established.

1790

Burtin, P. 1960. A study of serum proteins related to immunity and their cellular origin, p. 213-226. In Ciba Symposium of Cellular Aspects of Immunity. Little, Brown and Company, Boston.

The cellular origin of gamma and beta globulins was studied. It was assumed that beta 2 globulins arise in plasmocytes and other cells like the gamma globulins. Beta 2 globulins were localized in the spleen and ganglia.

1800

Chiappino, G.; Corbetta, L. 1962. The synthesis of gamma globulins in the human palatine tonsil: Immunohistochemical research with fluorescent antibodies. Arch. Ital. Otolig. Laringol. 78:6:864-894. In Italian.

FA was used to study palatine tonsils removed from patients between 5 and 55 years old. Synthesis of gamma globulin was studied. Modifications in the transition of gamma globulin were seen between infancy and adult and also between adult and advancing age. Gamma globulin was actively synthesized up to the pre-senile age. Antibodies produced by the germinal centers of the lymphatic follicles pass into the crypts.

1810

Coons, A.H. 1959. Some reactions of lymphoid tissues to stimulation by antigens, p. 113-129. In The Harvey Lectures, 1957-1958, Series LIII. Academic Press, New York.

A brief review is presented of the key facts about antibody formation, describing the main features of the antibody response as reflected in the serum and in the local lymph node. Fluorescent antibody technique is included as a method of obtaining morphological information concerning the fate of injected antigen and the detection of antibody in individual cells.

1820

Coons, A.H.; Leduc, E.H.; Connolly, J.M. 1955. Studies on antibody production: I. A method for the histochemical demonstration of specific antibody and its application to study of the hyperimmune rabbit. J. Exp. Med. 102:49-60.

The specific histochemical demonstration of antibody in cells and parts of cells is described. It is a two-stage immunological reaction on frozen sections of tissues: Reaction between antibody in the tissue and antigen applied in vitro, and the detection of areas where this antigen has been specifically absorbed by a precipitin reaction with fluorescein-labelled antibody. Fluorescence microscope examination reveals the yellow-green fluorescence where a precipitate has formed. A study of the hyperimmune rabbit on the first few days after the last of a series of intravenous antigen injections reveals that antibody against human globulin or ovalbumin is present in groups of plasma cells in the red pulp of the spleen, the medullary areas of lymph nodes, the submucosa of the ileum, and the portal connective tissue of the liver. Because of nonspecific reactions, the bone marrow could not be examined. Small amounts of antibody were occasionally visible in cells in the lymphoid follicles of the spleen and lymph nodes.

1830

Curtain, C.C. 1959. Possible sites of macroglobulin synthesis: A study with fluorescent antibody. *Australasian Ann. Med.* 8:143-150.

This paper describes experiments on the localization of macroglobulins by the fluorescent antibody technique in the tissues of two patients, one showing Mikulicz syndrome and the other suffering from myeloma. The first patient had the macroglobulin in large quantities in mature and immature plasma cells in the bone marrow. The macroglobulin was a product of a plasmacytosis reactive to the pathological process in the parotid gland. The second patient had both a macroglobulin and a myeloma globulin in his serum. Fluorescent antibody studies suggested that these two abnormal proteins originated in different plasma cells in the bone marrow that were, however, morphologically identical with orthodox staining. Neither protein could be demonstrated in frozen sections of other tissues obtained at autopsy.

1840

Dixon, F.J.; Weigle, W.O.; Vazquez, J.J. 1961. Metabolism and mammary secretion of serum proteins in the cow. *Lab. Invest.* 10:210-237.

Although the cow udder incorporates serum proteins, especially gamma globulin, into the lacteal secretions, the colostrum contains gamma globulin at 100 times the albumin concentration. Heterologous and autologous gamma globulins are concentrated similarly. The amount of gamma globulin in the colostrum approximately equals the amount that disappears from the serum at parturition. During the early part of the dry period, a hyper-gammaglobulinemia is present, but when the colostrum is formed, an antepartum hypogamma-globulinemia appears. Lactation loss of gamma globulin is about 10 per cent of the total gamma globulin loss normally, but may exceed catabolic loss during colostrum formation. The acinar epithelium of the udder apparently transports serum proteins, but little gamma globulin is formed in the udder. During the colostrum formation the acinar epithelium is a serum protein carrier, but during lactation it produces proteins.

1850

Engelhardt, G. 1958. The localization of antibody formation. *Deut. Med. Wochensch.* 83: 20:877-880. In German.

This is a broad review and comment on localization of antibody formation and the techniques used to study this phenomenon. As a portion of this paper, immunohistochemical studies are reviewed. Plasma cells were indicated as antibody-producing sites by this technique. Quantitation of antibody was not possible.

1860

Feldman, C.A. 1960. Cellular sources of gamma globulin. *Marquette Med. Rev.* 26:30-33

A brief review of the literature concerning cellular sources of antibody and gamma globulin has been prepared. Preliminary experimental procedures regarding the cellular sources of gamma globulin in the rabbit are described. Cells similar to the lymphocyte and plasma cell, together with their common precursor cell, contain and probably manufacture gamma globulin in the rabbit.

1870

Gitlin, D.; Craig, J.M. 1957. Variations in the staining characteristics of human fibrin. Amer. J. Pathol. 33:267-283.

Fibrin clots were prepared from purified fibrinogen and thrombin, either alone or with additional plasma proteins. Sections from these clots reacted specifically with fluorescein-labeled rabbit antihuman fibrin but reacted negatively when fixed, embedded in paraffin, sectioned, and stained by three independent standard histologic procedures: Mallory phosphotungstic acid-hematoxylin, Biebrich scarlet-aniline blue and Pearse periodic acid-leukofuchsin-hematoxylin-orange G. When fibrin clots were formed in the presence of albumin at a concentration greater than one gram per cent or glutathione at a concentration greater than 0.05 gram per cent, the resulting sections reacted positively for fibrin by the standard staining techniques. FA indicated the presence of fibrin in such clots. Dye-binding data and additional information are presented to suggest that the fibrin reacting positively with dyes and the fibrin reacting negatively with the same dyes represent two forms of the material that differ in their structural bonds or cross-linkages. The evidence suggests that the positively reacting form of fibrin is related to the urea-insoluble form of fibrin and that the negatively reacting form is related to the urea-soluble form of fibrin.

1880

Gitlin, D.; Landing, B.H.; Whipple, A. 1953. The localization of homologous plasma proteins in the tissues of young human beings as demonstrated with fluorescent antibodies. J. Exp. Med. 97:163-176.

Employing fluorescent antibodies for the detection of homologous plasma proteins in tissue sections, the distribution of plasma albumin, alpha-globulin, beta-lipoprotein, beta-1 metal-combining globulin, and fibrinogen has been studied in the tissues of infants and children. Plasma albumin, gamma globulin, and beta-1 metal-combining globulin were found in many cells and particularly in cell nuclei, connective tissues and interstitial spaces, lymphatics, and blood vessels. Beta-lipoprotein was found mostly in the nuclei of all cell types; fibrinogen was restricted largely to the lymphatic and vascular channels, connective tissues, and the interstitial spaces. The widespread distribution of these plasma proteins in cells and connective tissues indicates the magnitude of the extravascular plasma protein pool that is in equilibrium with circulating plasma. Unfortunately, these results do not permit accurate localization of the sites of production of these plasma proteins, but do give some idea of their intimate relationship to the tissues.

1890

Glass, L.E. 1959. Immunohistological localization of serum-like molecules in frog oocytes. J. Exp. Zool. 141:257-289.

Location of serum-like molecules in the intraovarian oocyte of the frog, Rana pipiens, was studied using fluorescent antisera to adult female frog serum employed as stains on sections of ovary containing oocytes at various stages of growth. Diffuse fluorescence was present in the cytoplasm of pre-yolk oocytes and was intense in the larger cells. In cells in early states of vitellogenesis, fluorescence was observed in bright masses at the periphery of the egg, as a matrix between yolk platelets, and as a perinuclear ring of yolk nuclei. In cells in late vitellogenesis, the bright peripheral clumps were absent. Nucleolar fluorescence was observed in some mature oocytes, although nucleoli

were nonfluorescent until late in yolk deposition. Fluorescence in follicle-thecal cells increased throughout vitellogenesis. Antiserum against saline-soluble oocyte antigens was localized on the nucleoli and at the sites of yolk deposition in oocytes undergoing vitellogenesis. In mature eggs, the antiserum was also localized in and around yolk platelets and in a narrow region of the cortex. The data suggest that part of the serum-like molecules visualized by the localization of fluorescent antibodies were large molecules synthesized elsewhere in the maternal body and transferred from maternal serum through the follicle-thecal cells and into the oocyte.

1900

Hamashima, Y.; Harter, J.G.; Coons, A.H. 1962. Cellular site of albumin and fibrinogen production in the human liver. Federation Proc. 21:304.

Human liver sections obtained by open biopsy at cholecystectomy were stained specifically with anti- β X crystallized human serum albumin, HSA, or antihuman fibrin monomer fluorescent conjugates. About 10 per cent of the hepatic cells stained for HSA, similar to staining for prothrombin reported by Barnhart. These cells tended to be more numerous around the central vein. Staining was predominately in the cytoplasm but nuclei were stained in many cells. About 20 per cent of the Kupffer cells also showed specific cytoplasmic staining for HSA. Cells staining for fibrinogen were more widely scattered and no definite lobular pattern was detected. Less than 1 per cent of the hepatic cells but about 70 per cent of the Kupffer cells stained for fibrinogen. In both cell types only cytoplasmic staining was observed. In addition, a line of small specifically staining granules could be seen just inside the cell membrane facing the bile canaliculi in many hepatic cells. The method of fixation of the liver sections is critical for the demonstration of these two antigens. So far, a suitable fixative has not been found for demonstrating albumin and fibrinogen in the same section, so that as yet it has not been possible to determine whether individual cells contain only one or both proteins.

1910

Heller, P.; Yakulis, V.J. 1960. Antigenicity of connective tissue extracts: II. Stimulation of auto- and iso-antibodies by heterologous antigen. Proc. Soc. Exp. Biol. Med. 104:590-594.

Tissue-fixed globulin has been demonstrated in interstitial tissue and basement membranes of various guinea pig organs following inoculation of guinea pigs with saline extracts of rabbit tendon. These animals had a significantly diminished growth. It is likely that the fixed globulin represents autoantibody induced by heterologous antigen.

1920

Horowitz, R.E. 1962. Gamma globulin in the lymphatic tissue of the germ-free mouse. Lab. Invest. 11:693-694.

The distribution of gamma globulin in the gastrointestinal tract lymph nodes, the systemic lymph nodes, and the spleen was studied in random germ-free and conventional mice by routine immunocytochemical techniques, and was correlated with quantitative morphology under the light microscope. Plasma cells showing specific fluorescence when stained with rabbit antimouse gamma globulin were numerous in the gastrointestinal tract lymph nodes

of the conventional mice but virtually absent in the germ-free mice. Such gamma globulin-containing cells were not found in the spleen. In lymph nodes anatomically unrelated to the gastrointestinal tract, there were rare plasma-cytoid cells showing specific fluorescence in the conventional but none in the germ-free. Although the number of plasma cells was lower in germ-free mice, the number of plasma cells with Russell bodies was greater. Absence of gamma globulin in lymphatic tissue of germ-free mice accompanies the known hypogammaglobulinemia that these animals demonstrate. This study indicates possible use of the germ-free animal in studies of distribution and fate of administered antigen and appearance and location of resultant antibody.

1930

Kaplan, M.H.; Craig, J.M. 1958. Production of cardiac lesions in rabbits immunized with heterologous heart tissue. Federation Proc. 17:2030:520.

Immunization of rabbits with homogenates of beef or rat heart stimulated antibodies reactive with normal rabbit heart tissue as demonstrated by immunofluorescence and complement fixation. The antigen has been identified as an alcohol-soluble organ-specific constituent of the sarcoplasm of striated muscle. It may be differentiated by cross-absorption tests from cardiacin antigen. Rabbits given repeated injections of beef or rat heart homogenate incorporated in either alumina gel or Freund adjuvant develop small focal cardiac lesions characterized by myofiber necrosis and or interstitial inflammation. Rarely, an extensive myocardial fibrosis has been observed. The results of one experiment were as follows: Cardiac lesions were noted in 8 of 8 animals injected with rat heart, 7 of 9 animals given beef heart, and in 3 of 13 control rabbits injected with human gamma globulin. Myofiber necrosis was noted in 12 of the 17 experimental animals and in one of the 13 controls. In 7 of 15 experimental animals, bound gamma globulin could be detected within the sarcoplasms of myofibers, although all of these 15 rabbits showed elevated levels of circulating antibody. Myocardial cells are resistant to penetration by antibody to the sarcoplasmic antigen. Resistance may represent a limiting factor in pathogenesis of cardiac lesions.

1940

Leduc, E.H.; Coons, A.H.; Connally, J.M. 1955. Studies on antibody production: II. The primary and secondary responses in the popliteal lymph node of the rabbit. J. Exp. Med. 102:61-72.

After an antigenic stimulus, antibody is first demonstrable in the cytoplasm, and often in a spot in the nucleus, of large, immature cells in the medullary areas of the lymph node draining the site of injection. Morphologically, these cells have basophilic cytoplasm and a large nucleus, and are typical hematogenous stem cells. As these cells multiply and differentiate, the concentration of antibody in their cytoplasm increases, until colonies of typical mature plasma cells containing antibody have developed. There is a marked difference between the primary and the secondary responses: the former is characterized by the development of a very few antibody-containing cells; in the latter there are hundreds in a similar area. The morphology of the cells involved in both responses is identical. Occasionally, antibody was also found in low concentration in association with the lymphoid follicles. The implications of these findings for an understanding of antibody synthesis are discussed.

1950

Lee, L.; McCluskey, R.T. 1962. Immunohistochemical demonstration of the reticuloendothelial clearance of circulating fibrin aggregates. *J. Exp. Med.* 116:611-618.

In rabbits given an intravenous infusion of thrombin or an injection of endotoxin, immunohistochemical examination of the tissues with conjugated antiserum against rabbit fibrin showed bright intracytoplasmic staining in many of the phagocytic cells of the liver and spleen. In normal rabbits as well as in animals injected with large doses of heparin prior to thrombin or endotoxin administration, no such intracellular staining was observed. The findings of this study substantiate the hypothesis that fibrin aggregates formed in the circulating blood during low-grade intravascular coagulation are largely removed by the reticuloendothelial system.

1960

Mancini, R.E.; Vilar, O.; Davidson, O.W.; Dellacha, J.M.; Gomez, O. 1961. Histological distribution of fluorescent albumin, globulins, and fibrinogen in the connective tissue of rats. *J. Histochem. Cytochem.* 9:634.

Fractions of normal rat plasma were labeled with fluorescent dye. Rats were injected with labeled or unlabeled fractions. Fluorescent rabbit antiserum conjugates were applied to tissue sections from injected animals. The disappearance of plasma proteins revealed a fast and a slow component. Labeled plasma proteins were in blood vessels, interstitial capsular and stromal connective tissue, perichondria, tendons, articular capsules, and basal membranes. They were most concentrated at 12 hours. Fibrinogen was in the vessels but did not diffuse. Proteins were not in parenchymatous cells. Proteins were in macrophages and cells of the convoluted tubules where there was a gradual accumulation. Labeled and unlabeled proteins had the same distribution, but the unlabeled ones, subsequently stained, yielded superior fluorescence.

1970

Mancini, R.E.; Vilar, O.; Davidson, O.W.; Heinrich, J.J.; Alvarez, B. 1962. Incorporation of labeled rat serum fractions by the rat ovary. *J. Histochem. Cytochem.* 10: 666.

Rat albumin, globulins, and fibrinogen were labeled with fluorescent dyes and radioisotopes. The amount of tracer bound to the protein and the electrophoretic mobility of the labeled serum fractions were studied. Normal adult female rats were injected with each of the fractions. The animals were sacrificed at different intervals of time and concomitantly blood samples were taken to study the concentration of the labeled fractions in the sera. The ovaries were processed for direct fluorescent microscopy and radioautography. An extravascular diffusion of the labeled proteins into the ovarian connective tissue was observed in close relation with the fast decay of these fractions in the blood. Only in the growing follicle were labeled proteins seen traversing the internal theca and basal membrane in the intracellular spaces reaching the antrum and the ovocyte. The proteins began to disappear from these localizations between 12 and 72 hours after injection with only slight differences between each two of the injected fractions. Fluorescent antibodies against rat albumin, globulins, and fibrinogen gave similar results when they were incubated with normal ovarian fresh-frozen sections.

1980

Mancini, R.E.; Vilar, O.; Dellacha, J.M.; Davidson, O.W.; Gomez, C.J.; Alvarez, B. 1962. Extravascular distribution of fluorescent albumin, globulin, and fibrinogen in connective tissue structures. *J. Histochem. Cytochem.* 10:194-203.

Rats, in groups, were intravenously inoculated with fluorescein isothiocyanate and rhodamine-labeled homologous albumin, globulin, and fibrinogen, and also free fluorescent dyes. Animals were sacrificed at intervals and tissues were examined immunohistologically. Studies on the reagents and experimental animals also included spectrophotometry and electrophoresis. Globulins bound the largest amount of dye, fibrinogen the least. Labeling did not grossly change electrophoretic mobility. Time concentration curves of albumin and globulin were similar, revealing fast and slow components. Albumin and globulin appeared first in the blood vessels, and then in connective tissues, dermis basement membranes, and digestive, respiratory, and orogenital mucosae. Fluorescence rarely appeared below the basement membranes, nor passed the perichondrium, periosteum, or nervous system blood capillaries. Disappearance of proteins started in 12 hours. Persistence was greatest in macrophages of spleen, marrow, liver, and lymph nodes. Albumin and globulins were located in connective tissue by indirect FA examination.

1990

McKay, D.G.; Gitlin, D.; Craig, J.M. 1959. Immunohistochemical demonstration of fibrin in the generalized Shwartzman reaction. *A.M.A. Arch. Pathol.* 67:270-273.

By use of the Coons and Kaplan fluorescent-antibody technique it has been demonstrated that fibrin or an insoluble derivative of fibrinogen is a constituent of the thrombi found in the lungs, spleen, liver, and kidneys in the generalized Shwartzman reaction. In combination with studies of the blood coagulation mechanism, it is concluded that fibrin is the essential constituent of these thrombi.

2000

Neil, A.L.; Dixon, F.J. 1959. Immunohistochemical detection of antibody in cell transfer studies. *A.M.A. Arch. Pathol.* 67:643-649.

In transfers of lymph node cells, the time and magnitude of the antibody response and the immunochemical characteristics of the antibody in the recipient indicate that the transferred cells make the antibody. Since the antibody can be localized within developing plasma cells in the cell transfer site, it appears probable that the plasma cells are derived from the transferred lymph node cells. Considering the minimal mitotic activity in the transfer sites, it appears that during their antibody response the transferred lymphocytes metamorphose to the plasma cells without division by the stages described.

2010

Ortega, L.G.; Mellors, R.C. 1957. Cellular sites of formation of gamma globulin. *Amer. J. Pathol.* 33:614.

Fluorescent antibody was prepared against human gamma globulin. Specificity-blocking controls on tissue sections were employed. Normal and moderately hyperplastic lymph

nodes and spleens, spontaneous cancers, homotransplanted cancers, and connective tissue lesions, all human, were studied. Gamma globulin was localized in plasma cells with smooth or granular cytoplasmic staining, the cytoplasm of these cells being basophilic in H and E sections; in plasma cells containing globular cytoplasmic bodies with surface staining, these containing Russell bodies under H and E or PAS stain; and in primitive reticular cells of the germinal center of lymph follicles having large nuclei and scanty, finely granular cytoplasm with stellate cytoplasmic processes. Staining occurred only in the granules.

2020

Ortega, L.G.; Mellors, R.C. 1957. Cellular sites of formation of gamma globulin. J. Exp. Med. 106:627-640.

The cellular sites of formation of gamma globulin in lymphatic tissues of man and in a representative human lymphoid infiltrate have been studied by FA. Gamma globulin is formed in the germinal centers of lymphatic nodules and in the cytoplasm of plasma cells with and without Russell bodies. The germinal center cells that synthesize gamma globulin have been designated intrinsic cells to distinguish them from the medium and large lymphocytes and from the primitive reticular cells. Unlike the plasma cells, which function as individual units or discrete aggregations, the function, blood supply, and systematic cellular arrangement of germinal centers justify the postulate that they are miniature organs of internal secretion of gamma globulin. The release of gamma globulin from its sites of formation appears to be accomplished by holocrine and apocrine secretion, adaptations required for the production of antibody. Cells found to form gamma globulin appear identical with those shown to form specific antibody. Normal gamma globulin, if it exists, originates in the same cells that produce antibody. Each of the three morphologically distinct categories of cells that synthesize gamma globulin may represent a response to a particular form of antigenic stimulation. Nuclear participation in gamma globulin synthesis was not detected.

2030

Payne, L.C.; Marsh, C.L. 1962. Absorption of gamma globulin by the small intestine. Federation Proc. 21:909-912.

The pig was used to study protein absorption by tracing with fluorescein isothiocyanate-labeled gamma globulin. Within a few hours after exposure of absorptive cells to soluble protein, an active absorption state converts to a complete nonabsorptive state. The use of protein-free diets may prolong the period of active absorption.

2040

Rappaport, B.Z. 1960. Antigen-antibody reactions in allergic human tissue: II. Study by fluorescence technique of the localization of reagins in human skin and their relation to globulins. J. Exp. Med. 112:725-734.

The skin of atopic patients contains specific reaginic antibody in all epidermal cells, in unchallenged as well as in antigen-challenged areas. The reagins are also present in the epithelial cells of sebaceous glands, sweat glands, hair follicles, and in macrophages and pericytes. Judged by staining characteristics, their immunologic reaction in tissues

with antigen is identical with that of rabbit antibodies. Comparison of tissues stained with conjugated reagins and conjugated antihuman globulin antibodies demonstrated the close relationship of reagins and globulins. In antigen-challenged tissues the macrophages and pericytes become enlarged and stain more intensely with toluidine blue. No such changes in morphology or staining are present in histamine-tested or unchallenged atopic tissues.

2050

Roberts, A.N.; Haurowitz, F. 1962. Intracellular localization and quantitation of tritiated antigens in reticuloendothelial tissues of mice during secondary and hyperimmune responses. *J. Exp. Med.* 116:407-422.

Fluorescent labeled anti-arsanilazo porcine gamma globulin was used to demonstrate antibody-synthesizing cells in sections of regional lymph nodes following footpad injections of H₃-arsanilazo porcine gamma globulin. This correlated with radioactive measurements.

2060

Vazquez, J.J. 1961. Antibody or gamma globulin forming cells, as observed by the fluorescent antibody technic. *Lab. Invest.* 10:1110-1125.

Fluorescent antibody studies have helped establish the view that plasma cells are the major antibody-forming cells. Apparently, plasma cells differentiate from a primitive cell on antigenic stimulation. This study reports a preliminary study on the mechanism of cell antibody release.

2070

White, R.G. 1960. The relation of the cellular responses in germinal or lymphocytopoietic centers of lymph nodes to the production of antibody, p. 25-29. In *Mechanisms of antibody formation*. Publishing House of the Czechoslovak Academy of Sciences, Prague.

A primary stimulus of antigen causes focal lymphocytogenesis in the regional lymph nodes. These cells then attain the capacity to react to further antigen stimulus. Lymphocytes produced in these centers could be the morphological basis for the primary response.

2080

White, R.G.; Coons, A.H.; Connolly, J.M. 1953. Cellular morphology of antibody production: The alum granuloma. *Federation Proc.* 12:465-466.

Antibody-containing cells have been visualized under the fluorescence microscope by using frozen sections treated with homologous antigen and fluorescein-conjugated antibody. Alum-precipitated antigens - egg albumen and diphtheria toxoid - were injected subcutaneously into the footpad of rabbits and guinea pigs, and histological studies were confined to the resulting local granuloma and the regional lymph gland. The local granulomata of the rabbit contained a high concentration of cells with the morphology, as checked by Giemsa and hematoxylin- and eosin-stained preparation, of hemocytoblasts, immature and mature plasma cells, which showed a high content of antibody as indicated by this staining method. The macrophages of the granulomata were readily identified under the fluorescence microscope

by their content of pale blue granules of aluminum hydroxide. These cells were devoid of antibody staining. Antibody-containing cells were also present in the regional lymph node. In the guinea pig, a greater proportional content of antibody-containing cells existed in the regional lymph node and a lesser in the local granuloma. In both the rabbit and the guinea pig, the appearance of isolated cells at different stages of morphological differentiation within the same regional lymph node draining an alum granuloma contrasted with the uniformity of cell types present in nodes following either a normal primary or secondary stimulation with soluble antigen. Complete article.

2090

White, R.G.; Coons, A.H.; Connolly, J.M. 1955. Studies on antibody production: III. The alum granuloma. *J. Exp. Med.* 102:73-82.

After subcutaneous injection of hen ovalbumin or diphtheria toxoid precipitated with aluminum phosphate, the production of antibody, as judged by the presence in the tissues of antibody-containing cells proceeds partly within the regional lymphatic glands and partly in the granulation tissue surrounding the nodule that develops at the site of injection. The first production of antibody takes place in the regional lymphatic gland, and antibody production in the local granuloma becomes apparent only after 14 days in the rabbit. Antibody-containing plasma cells were demonstrated in the local granuloma up to 7 weeks. Antibody-containing cells in the regional lymphatic glands reach maximum numbers at 2 weeks following injection and decrease thereafter to a few cells at 5 weeks. The adjuvant effect of the aluminum phosphate is interpreted as due partly to the delay in absorption of antigen from the local site of its injection, which results in prolongation of stimulation of cells within the regional lymphatic glands, and partly to the production of a local granuloma that contains antibody-producing plasma cells.

2100

Wollensak, V.J.; Seybold, G. 1957. Demonstration of serum proteins in liver and kidney by fluorescent antibody. *Z. Naturforsch.* 12B:147-150. In German.

Traces of species-specific albumin were demonstrated in the parenchymal cells of the liver in comparative studies of the livers of man, rat, and guinea pig. Considerable albumin was in the Kupffer star cells. Human gamma globulin could be demonstrated in the human liver only in Kupffer star cells. These proteins were stored in the liver in the star cell protoplasm after dispensation of human albumin and human gamma globulin to rats and guinea pigs. The glomerular stigmata of rat and guinea pig kidneys failed to reveal storage of homologous albumin. Even after dispensation of human albumin and human gamma globulin, the heterologous proteins could not be demonstrated in these cells. No rat albumin was found in the tubular cells of the rat kidney in spite of protein urea. Vital storage of trypan blue and lithium carmine does not change the distribution of homologous liver serum albumin in rats and guinea pigs.

2110

Woolf, N. 1961. The distribution of fibrin within the aortic intima. Amer. J. Pathol. 39:521-529.

The application of fluorescent antibody to a study of the distribution of fibrin in the aortic intima is described. The amount of fibrin increases with intimal thickness, and the presence of atherosclerotic lesions of various types is associated with a further marked increase. Differing patterns of distribution suggest that infiltration of plasma fibrinogen as well as mural deposition and incorporation may be responsible for the presence of the protein within the intima. Differing emphasis on these mechanisms among different population groups might be partly responsible for epidemiologic differences.

2120

Woolf, N.; Crawford, T. 1960. Fatty streaks in the aortic intima studied by an immuno-histochemical technique. J. Pathol. Bacteriol. 80:405-408.

Frozen sections of unfixed human aortae were treated with a fluorescein-coupled rabbit antihuman-fibrin serum to identify fibrin. Specific fluorescence was consistently observed in superficial fatty streaks.

B. PATHOLOGY

2130

Becker, B.; Coleman, S.L. 1962. Gamma globulin in ocular diseases: Diabetes and glaucoma. *Trans. Amer. Ophthal. Soc.* 60:260-267.

Human eyes have been studied after staining with fluorescein-labeled antihuman gamma globulin. In diabetic eyes the thickened basement membrane of the ciliary body and the capillary aneurysms of the retina stained intensely. Eyes with chronic open-angle glaucoma demonstrated remarkable staining of their trabecular meshworks.

2140

Becker, B.; Keates, E.U.; Coleman, S.L. 1962. Gamma globulin in the trabecular meshwork of glaucomatous eyes. *Arch. Ophthalm.* 68:643-647.

Direct antihuman gamma globulin FA was used to study the trabecular meshworks of glaucomatous and normal autopsy eyes. Almost two-thirds of the glaucomatous eyes demonstrated abnormal positive reactions indicating gamma globulin at the site of increased resistance to aqueous outflow.

2150

Cohen, S.; Ohta, G.; Singer, E.J.; Popper, H. 1960. Immunocytochemical study of gamma globulin in liver in hepatitis and post-necrotic cirrhosis. *J. Exp. Med.* 111:285-294.

Gamma globulin was demonstrated by immunocytochemical fluorescence technique in many reticuloendothelial cells of the hepatic sinusoids and of the fibrous tracts in various forms of hepatitis and in post-necrotic cirrhosis. In other liver diseases and in normal liver, even in the presence of hypergammaglobulinemia, few if any gamma globulin-containing cells were found. Spleen and lymph nodes showed no difference between post-necrotic cirrhosis or hepatitis and other types of cirrhosis or non-hepatic hypergammaglobulinemias. The gamma globulin-containing cells in the liver are, on cytologic grounds, considered reticuloendothelial cells showing transition to plasma cells and exhibiting little or no phagocytosis of tissue breakdown products. These cells are assumed to form rather than engulf gamma globulin. The possibility that the gamma globulin formed represents antibody to liver cell breakdown products is discussed.

2160

Corbetta, L.; Chiappino, G. 1962. Application of fluorescent antibody technique to the study of the histopathological changes induced by nasal ozena. *Ann. Laringol. Otol. Rinol. Faringol.* 61:6:687-691. In Italian.

Autoantibody globulins in the serum and nasal mucosa of patients with ozenatous atrophic rhinitis were studied. In ozena cases it was rarely possible to find gamma globulin with affinity for nasal mucosa. Ozenatous nasal mucosa was always negative for gamma globulin.

2170

Crawford, T.; Woolf, N. 1960. Hyaline arteriolosclerosis in the spleen: An immuno-histochemical study. *J. Pathol. Bacteriol.* 97:221-225.

Unfixed frozen sections of human spleens showing hyaline arteriolosclerosis were treated with a fluorescein-coupled rabbit antiserum to human fibrin. Specific fluorescence was constantly observed in the hyalinized intima of affected vessels. Negative results were obtained in spleens from infants and children who showed no lesions when examined by ordinary methods. The specificity of the antifibrin serum was established by precipitation, blocking, and absorption techniques. Empirical staining for fibrin gave much less constant positive findings. These findings in toto lend support to the encrustation hypothesis for the pathogenesis of splenic hyaline arteriolosclerosis.

2180

Cruchaud, A.; Rosen, F.S.; Craig, J.M.; Janeway, C.A.; Gitlin, D. 1962. The site of synthesis of the 19S gamma globulins in dysgammaglobulinemia. *J. Exp. Med.* 115:1141-1148.

Lymph nodes and splenic tissue from patients with congenital agammaglobulinemia and dysgammaglobulinemia and from normal subjects were studied with immunofluorescence and histochemical stains to determine the site of synthesis of the 19S gamma globulins. The two patients with dysgammaglobulinemia had high serum concentrations of the 19S gamma globulins and a marked deficit of the 7S gamma globulins. These patients, as well as agammaglobulinemic children, had only rare or no plasma cells in their tissues. Cells were identified in sections of spleen from a dysgammaglobulinemic child as well as from normal individuals who exhibited specific fluorescence with an anti-19S gamma globulin antiserum adsorbed with 7S gamma-2-globulins that stained positively with PAS and methyl green pyronine. These cells resembled the transitional cells described by Fagraeus.

2190

Dutcher, T.F.; Fahey, J.L. 1960. Immunocytochemical demonstration of intranuclear localization of 18S gamma macroglobulin in macroglobulinemia of Waldenstrom. *Proc. Soc. Exp. Biol. Med.* 103:452-455.

Immunofluorescent and cytochemical techniques were used to identify 18S gamma macroglobulin within the nucleus and cytoplasm of lymphocytoid plasma cells from a patient with macroglobulinemia of Waldenstrom. Intranuclear fluorescence coincided with presence of intranuclear PAS-positive protein. Circulating macroglobulin and intranuclear protein of lymphocytoid plasma cells are closely related and may be identical. The evidence suggests that macroglobulin is formed in lymphocytoid plasma cells.

2200

Gitlin, D.; Craig, J.M. 1956. The nature of the hyaline membrane in asphyxia of the newborn. *Pediatrics* 17:64-71.

The lungs of five newborn infants who succumbed because of respiratory difficulty with the formation of pulmonary hyaline membranes were studied by fluorescent antibody and dye-staining procedures. Human amniotic fluid was examined by the same methods. The

hyaline membranes in these infants were composed largely of fibrin. Although small amounts of fibrin were found in some of the amniotic fluids studied, the quantities were small compared with those found in the membranes. It was concluded that hyaline membranes can be produced as the result of effusion from the pulmonary circulation; conversion of fibrinogen in the effusion to fibrin, which would be enhanced by the thrombo-plastic activity of aspirated amniotic fluid; and syneresis of the fibrin to form a membrane. It is recognized that such fibrin-containing membranes are not solely composed of fibrin but also contain other elements such as lipid and squamous cells from vernix in the amniotic fluid.

2210

Goldwasser, R.; Rozansky, R. 1961. Detection of C-reactive protein by fluorescent antibody techniques. *Nature* 190:1020-1021.

The presence of C-reactive protein in human sera is a highly sensitive index of inflammation or of tissue destruction. The methods available for the detection of C-reactive protein are based on precipitation, complement-fixation, hemagglutination, and gel-diffusion techniques. Results of fluorescent antibody-stained blood smears from people positive and negative for C-reactive protein with the fluorescent antibody techniques are described.

2220

Kaplan, M.H. 1959. The fluorescent antibody technique as a research tool in the study of connective tissue disease. *Arth. Rheum.* 2:568-573.

This method permits a descriptive analysis of pathologic lesions in terms of the variety of serum proteins or tissue antigens present and has the potential of permitting the detection of serologic reactivity of autoimmune factors in the serum of gamma globulins deposited in the tissues. The identification on a purely descriptive level of the serum protein constituents of pathologic lesions has permitted observations of certain associations that may be pathogenetically meaningful, e.g., gamma globulin and fibrinoid, fibrin and fibrinoid, 7S and 19S gamma globulin. With our present information, the significance of such descriptive associations cannot be stated. The major problem to be clarified seems to be the nature and function of the gamma globulin deposited in rheumatic lesions. Whether such gamma globulin, 7S and 19S, represents totally or in part antibody to exogenous or endogenous material, or whether it has no immune function at all, is a problem confounded by our ignorance of the immunology of such systems and by the perplexing nature of the alterations of connective tissue in rheumatic lesions. Fluorescent antibody as a research tool may prove valuable.

2230

Koffler, D.; Garlock, J.; Rothman, W. 1962. Immunocytochemical reactions of serum from ulcerative colitis patients. *Proc. Soc. Exp. Biol. Med.* 109:358-360.

Increased gamma globulin was demonstrated in the intestinal tract of patients with active regional ileitis and ulcerative colitis. The indirect fluorescent antibody technique revealed a gamma globulin in the serum of 3 of 25 patients with ulcerative colitis that was bound to epithelial glands of colonic mucosa and of hepatic bile ductules. Sera of normal and hospital controls did not bind to colonic mucosa.

2240

Koffler, D.; Woolf, N. 1962. Immunocytochemical studies in ulcerative colitis and regional ileitis. Federation Proc. 21:15.

Sera of patients with ulcerative colitis and regional ileitis were studied by indirect fluorescent antibody. Circulating gamma globulins binding to normal adult colonic epithelium were found in 5 of 27 patients with ulcerative colitis. Similar reactions were noted with bile ductules of a liver showing active malignant hepatitis. Sterile fetal colon, benign polyps, and carcinoma of the colon were stained only with sera that demonstrate circulating antibody in order to further elucidate the nature of the antigenic substrate. Fluoresceinated rabbit antisera to various fractions of human serum were employed to demonstrate gamma globulin, albumin, fibrinogen, macroglobulin, and complement distribution in segments of intestine affected by granulomatous ileitis and ulcerative colitis. An increased amount of gamma globulin was found in the mucosa and the submucosa of these specimens. These findings suggest that antibodies to colonic epithelium are formed in some patients with ulcerative colitis, and the gamma globulin is formed in increased quantities or deposited in areas of acute inflammatory disease of the small and large intestine.

2250

Ohta, G.; Cohen, S.; Singer, E.J.; Rosenfield, R.; Strauss, L. 1959. Demonstration of gamma globulin in vascular lesions of experimental necrotizing arteritis in the rat. Proc. Soc. Exp. Biol. Med. 102:187-189.

Experimental necrotizing arteritis was produced in female Sprague-Dawley rats by administration of large doses of desoxycorticosterone acetate and sodium chloride after unilateral nephrectomy. Presence of rat gamma globulin in damaged vessel walls was demonstrated by FA. Gamma globulin is deposited in smooth muscle of damaged small arteries, especially in early phases of vascular damage, suggesting that antibody may not be required for this process.

2260

Ohta, G.; Cohen, S.; Zak, F.G.; Singer, E.J. 1959. Immunohistochemical studies on spleen and liver. Federation Proc. 18:1959:497.

Spleens from autopsied patients with hypergammaglobulinemia were examined for the localization of gamma globulin by FA. Several cell types contain gamma globulin: plasma cells, plasmacytoid cells, several stages in the development of plasmacytoid cells from histiocytes, and large mononuclear cells or splenic tumor cells. In hyperglobulinemia the distribution of gamma globulin in the cells of the follicle was the same as in the normal spleen despite the difference in cytology. Occasionally, lipofuscin and gamma globulin were in the same cell, just as in the liver. Despite great variation in proportion of types of cells and their gamma globulin content, spleens in chronic splenitis showed gamma globulin mainly in plasma cells, spleens in liver cirrhosis in the plasma or plasmacytoid cells, and in acute splenitis, in the splenic tumor cells. The relationship of the splenic gamma globulin distribution to the previously reported increase in pyroninophilia of splenic pulp cells in liver cirrhosis and to hepatic alteration is under investigation.

2270

Paronetto, F.; Rubin, E.; Popper, H. 1962. Local formation of gamma globulin in the diseased liver, and its relation to hepatic necrosis. *Lab. Invest.* 11:150-158.

Immunocytochemical investigations indicated the presence of gamma globulin in all types of cirrhosis; in hepatitis, in addition to fibrinogen, albumin and other plasma proteins were demonstrated, implicating phagocytosis only in acute conditions. Gamma globulin was not eluted at pH 3.2, suggesting that it is not part of an antigen-antibody complex. In cirrhosis the presence of mesenchymal cells containing gamma globulin was not correlated with alcoholic tissue injury or with ischemic or biliary necrosis. It did correlate with piecemeal necrosis, characterized by disappearance of liver cells on the lobular or nodular periphery, the accumulation of lymphocytes, plasma cells, and histiocytes, and the proliferation of bile ductules. This type of necrosis was previously considered the morphologic expression of the progression of cirrhosis independent of its type.

2280

Shorter, R.G.; Lack, R.G. 1961. Immunological study of experimentally induced extrahepatic cholestasis. *Nature* 191:610-611.

Fluorescent antibody was used to examine livers damaged experimentally. The various cells involved were examined for gamma globulin. It was thought doubtful that the increase in hepatic gamma globulin in some liver diseases was immuno-specific. A unitary concept of liver disease is possible because the appearance of gamma globulin in reticuloendothelial cells lining sinusoids is common to a variety of hepatic conditions.

2290

Vazquez, J.J.; Dixon, F.J. 1956. Immunohistochemical analysis of amyloid by the fluorescent technique. *J. Exp. Med.* 104:727-736.

The immunohistochemical composition of amyloid deposits in secondary human amyloidosis and experimental amyloidosis in rabbits was studied by the fluorescent antibody technique. Quantitative studies of the relative amounts of gamma globulin present in the amyloid deposits by the use of radiodinated fluorescent antibody are reported. Amyloid deposits in several organs from cases of secondary human amyloidosis and experimental amyloidosis in rabbits contain considerable concentrations of gamma globulin. The presence of gamma globulin in amyloid might be interpreted as either a metabolic deposition of circulating globulin present in high concentrations in the plasma or as a result of an immunologic reaction involving antigen and antibody.

2300

Walzer, R.A. 1962. Case for diagnosis: Hypersensitivity reaction to propylthiouracil with immunoleukopenia. *Arch. Dermatol.* 86:170-171.

As a portion of this study FA demonstrated a globulin in patient serum that reacted with cytoplasm of polymorphonuclear leukocytes. This factor was not seen in normal and pathological controls.

2310

Wise, L.J.; Shames, J.M.; Derbes, V.J.; Hunter, F.M. 1961. Fluorescent antibody studies in chronic dermatitis. Arch. Dermatol. 84:37-39.

Skin biopsy specimens from 14 patients were studied with fluorescent antibody. Labeled serum was reacted with autologous skin. In two patients globulins were found with a specific affinity for dermotic skin, but they were nonreactive against skin not affected by the dermatitis.

V. BASIC IMMUNOLOGY

A. ANTIBODY IN SITU

2320

Coons, A.H.; Leduc, E.H.; Kaplan, M.H. 1951. Localization of antigen in tissue cells: VI. The fate of injected foreign proteins in the mouse. *J. Exp. Med.* 93:173-188.

The fate of three proteins, crystalline chicken egg albumin, crystalline bovine plasma albumin, and human plasma alpha globulin, was traced after intravenous injection into mice. This was done by preparing frozen sections of quick-frozen tissue, allowing any foreign protein that might be present in the section to react with homologous antibody labeled with fluorescein, and examining the section under the fluorescence microscope. By this means, which employs the serological specificity of the proteins as a natural marker, all three of these proteins were found in the cells of the reticuloendothelial system, the connective tissue, the vascular endothelium, the lymphocytes of spleen and lymph node, the epithelium of the kidney tubules, the liver, and in very small amounts in the adrenal. The central nervous system was not studied.

2330

Hamashima, Y.; Hayashi, K.; Takahashi, S.; Kyogoku, M.; Kitasawa, T. 1955. Immuno-cytological studies employing labeled active protein: I. Disposition of fluorescent active conjugate by the living body. *Acta Sch. Med. Univ. Kioto* 32:123-137.

The active protein was conjugated with aminofluorescein through the linkage of lysine radicals of the carbamido-group without destroying its capacity to react specifically with its immunological opponent. The frozen sections containing the active protein in the cells were prepared by applying low temperature. The time and degree of appearance of conjugate protein in the tissue cells of normal mice were studied. Certain cells were found to be activated in the sensitized mice: Kupffer cell, endothelium of hepatic sinusoid, the cells in the sensitive zone around the splenic and lymphatic follicles, capillary endothelium around alveoli, and the endothelium lining the blood vessels of the liver, spleen, adrenal, and lungs.

2340

Kaplan, M.H. 1958. Immunologic studies of heart tissue: I. Production in rabbits of antibodies reactive with an autologous myocardial antigen following immunization with heterologous heart tissue. *J. Immunol.* 80:254-267.

Inoculation of rabbits with whole streptococcal cultures grown in beef heart medium stimulated antibody reactive with normal rabbit heart sections, as determined by a modified immunofluorescent technique. The immunofluorescent reactivity of the serum was correlated with complement-fixing antibodies to normal rabbit heart tissue suspension. This antibody response was not related to the streptococcus but to the beef heart medium in which the streptococci were grown. Immunization with beef heart medium alone or with homogenate of beef heart also elicited both types of antibody. Similar results were obtained following immunization of rabbits with homogenates of rat and human heart. These data give evidence of a cross-reacting myocardial antigen in the sarcoplasm of the several mammalian species tested.

2350

Marshall, A.H.E.; White, R.G. 1961. The immunological reactivity of the thymus. Brit. J. Exp. Pathol. 42:379-385.

It has been shown that direct injection of antigens into the thymus of guinea pigs induces the formation of germinal centers and plasma cells and the production of antibody. The guinea pig thymus possesses a barrier against the entry of vital dyes or antigens from the blood stream into the gland that may be broken down by local trauma. The significance of these findings is discussed, especially in relation to the pathogenesis of myasthenia gravis.

2360

Mayersbach, H.; Pearse, A.G.E. 1956. The metabolism of fluorescein-labeled and unlabeled egg white in the renal tubules of the mouse. Brit. J. Exp. Pathol. 37:81-89.

The tubular absorption of foreign protein and the intracellular development of specific droplets are reported. Mice were given injections of egg white, either unlabeled or fluorescein-labeled, and the kidneys, freeze-dried or fixed by conventional methods, were examined by three different techniques. Tracing by fluorescent marker and tracing by fluorescent antibody showed good agreement with each other but little agreement with tracing by conventional staining or histochemical techniques for protein. Egg white, passing through the glomeruli, is rapidly absorbed by the proximal convoluted tubules. It is equally rapidly broken down to smaller products, and ultimately these return via the basement membrane to the blood stream. In the later stages of absorption the fluorescent label appears in the cytoplasm of the distal convoluted tubules. This may indicate uptake from the lumen or from the interstitial tissues. By neither of the fluorescent methods employed could positive evidence for the presence of whole egg white in the droplets be obtained.

2370

Nagai, H.; Nakano, H. 1958. Contribution of the study of intracellular antigens and antibodies. Arch. Franc. Pediat. Paris 15:160-182. In French.

Fluorescein isocyanate was conjugated to various antigens, their distribution traced, and immunogenicity studied. Following injection into mice and guinea pigs, the antigens were most heavily concentrated in the liver and spleen, and the fluorescence persisted there the longest. In previously sensitized animals the fluorescent reaction upon injection was immediate but showed less cellular persistence when compared with that of nonsensitized animals. The major portion of fluorescence augmentation from presensitization seemed due to pre-existent cells rather than newly formed cells, causing increase in fluorescence and augmentation of phagocytosis. Fluorescent antibodies forming antigen-antibody complexes were used to study antibody within cells. Tissues were prepared by freeze-drying, freezing in a deep freeze, or freezing by immersion. Casein was used for an antigen, and results indicated that antigen was rapidly eliminated by contact with the antibody. Therefore, antigens have been located intracellularly by the fluorescent antigen method but not clearly by the fluorescent antibody method.

2380

Neil, A.L.; Dixon, F.J. 1958. Immunohistochemical detection of antibody in cell transfer studies. Federation Proc. 17:2060:527.

A lymph node cell suspension of lymphocytes, with a few reticuloendothelial and plasma cells, from donors immunized with bovine serum albumin, BSA, was transferred intramuscularly and subcutaneously to X-irradiated recipient rabbits and the recipients were then challenged with BSA labeled with iodine 131. Circulating BSA was eliminated 5 days after transfer and maximum circulating anti-BSA was present 4 days later. The recipients were sacrificed 3 through 9 days after transfer, and tissue from the cell transfer site was obtained for study by FA. The morphology of antibody-containing cells was studied by staining with H and E stain the frozen tissue sections previously used in the fluorescent technique. Antibody-containing cells were observed 3 days after transfer. Cells resembled immature lymphoid or preplasma cells, and the maximum number was identified 5 to 6 days after transfer, located around large and medium-sized blood vessels and widely distributed between muscle fibers. The more intensely stained cells were similar to mature plasma cells. Less intensely stained immature forms were also present. Nine days after transfer the number of antibody-containing cells, now virtually all plasma cells, was reduced. Cytoplasm of positive cells was diffusely stained, and only rarely did we observe positively stained cytoplasmic globules suggestive of Russell bodies.

2390

Pressman, D.; Yagi, Y.; Hiramoto, R. 1958. Comparison of fluorescein and iodine 131 as labels for determining in vivo localization of anti-tissue antibodies. Int. Arch. Allergy Appl. Immunol. 12:125-136.

The fixation of localizing anti-tissue antibodies in vivo as determined by fluorescein-labeled antibody stain techniques and by radioactive techniques are compared. The fluorescein stain technique has the advantage of histological precision, i.e., localization of antibody may be determined on a cellular basis. However, a large concentration of antibody must be present for detection. The precision of localization as determined by radioautographs with iodine 131 is less than that by FA, but location of most of the smaller amounts of antibody is possible. Both techniques have the advantage that they can demonstrate a small amount of antibody in the presence of a large amount of background protein. Other advantages of the radic label are discussed. The direct injection of FA has no merit because the amount required for localization is larger than that required for FA staining and the labeled antibody nonspecifically localizes in the liver.

2400

Spear, G.S. 1962. Forssman antigen in the guinea pig: A histologic study. Bull. Johns Hopkins Hospital 111:252-265.

Forssman antigen was detected in the kidneys, heart, and lungs of normal guinea pigs by FA and phase contrast. The antigen was particulate. The distribution pattern was constant, but exact localization was not obtained.

2410

Tanaka, N.; Leduc, E.H. 1956. A study of the cellular distribution of Forssman antigen in various species. *J. Immunol.* 77:198-212.

The fluorescein-labeled antibody technique was employed in the localization of Forssman antigen in the tissues of guinea pig, cat, dog, mouse, and chicken. Antisera to sheep blood cells and to horse kidney were prepared in rabbits. The globulin fractions of the sera were labeled with fluorescein isocyanate. The specificity of the stain was established by several methods. The Forssman antigen was found as droplets in the endothelium and adventitial connective tissues of the blood vessels in all organs of all species examined. Species differences in the distribution of Forssman antigen in other tissues are described.

2420

Vainio, T.; Saxen, I.; Toivonen, S. 1960. Transfer of the antigenicity of guinea pig bone marrow implants to the graft tissue in explantation experiments. *Experientia* 16: 27-29.

As a portion of the reported work, fluorescent antibody methods demonstrated visually the passage of bone marrow antigenicity into presumptive epidermis of the common newt when used in a sandwich explant technique.

2430

Waksman, B.H.; Bocking, D. 1953. Study with fluorescent antibody of fate of intradermally injected proteins in rabbits. *Proc. Soc. Exp. Biol. Med.* 82:738-742.

In the rabbit, intradermally injected crystalline egg albumin and bovine gamma globulin are taken up by histiocytic cells both locally and in the peripheral and medullary sinuses of the draining lymph node. Considerable quantities remain extracellular and presumably diffuse to be dealt with elsewhere in the body. Polymorphonuclear cells, though present in considerable numbers, are not found to contain intact antigen. In the draining node, many lymphoid cells containing antigen are seen in the peripheral portions of the lymphoid nodules. Egg albumin disappears much more quickly than bovine gamma globulin from the injected skin site and the draining node. Passive sensitization and to a greater extent sensitization with Freund adjuvants result in a slowing of the disappearance of both antigens from skin and node. Adjuvant-sensitized animals also show speeding and intensification of phagocytosis of antigen in both localities.

2440

White, R.G. 1954. Observations on the formation and nature of Russell bodies. *Brit. J. Exp. Pathol.* 35:365-367.

Cytoplasmic bodies appear in mature plasma cells in the spleen of rabbits and mice following repeated intravenous injection of various bacillary vaccines. These bodies resemble Russell bodies in morphology and staining reactions. Russell bodies appear at 3 days and are at a maximum in 7 to 9 days in mice following recall antigenic stimulus. Intracytoplasmic crystals developing in a mature plasma cell have the same staining reactions as Russell bodies. The surface of Russell bodies produced in mouse spleen by *P. vulgaris* injections stained intensely when treated with anti-*P. vulgaris* serum. Therefore, specific antibody is a major component of Russell bodies.

B. ANTIGEN-ANTIBODY COMPLEXES

2450

Bennett, J.C.; Haber, E. 1962. Antigen conformation during dissociation of antigen-antibody complexes. *Arth. Rheum.* 5:637.

During studies on reoxidation and refolding of reduced antibody and its fragments, it has become necessary to insure the production of highly purified antibody that is completely free of antigen. By the use of fluorescent labeling of the antigen molecule, the amount of antigen contaminating any antibody preparation can be easily quantified. This technique can be used to show that the usual methods for dissociation of immune complexes such as acid, urea, salts, haptens excess, or detergents are not uniformly reliable for antibody purification. However, in the fluorescent ribonuclease-antiribonuclease system, complete dissociation can be achieved by conditions that produce an uncoupling of the alpha helix of ribonuclease. This conformational change, which occurs with heat in 1.0 M acetic acid, can be analyzed by optical rotatory methods. The conditions employed are not sufficient to produce an alteration of antibody structure. Antibody, which is 95 per cent precipitable, can then be separated in 90 per cent yield on heated columns of Sephadex G-100.

2460

Cochrane, C.G.; Weigle, W.O. 1958. Cutaneous reaction to soluble antigen-antibody complexes. *Federation Proc.* 17:1980:507.

BSA-rabbit and anti-BSA complexes, prepared by dissolving washed immune precipitates in excess antigen, were injected intradermally into rabbits. Doses of 0.5 mg antibody N produced erythema and edema, and with doses up to 2.4 mg antibody N, necrosis was also observed. Microscopically, the reaction showed a diffuse infiltration of polymorphs with vascular margination and plugging and also necrosis of occasional vessels by 24 hours. Using fluorescent antibody, BSA antigen was found scattered in the dermis. Vessels, including those showing margination and diapedesis of leukocytes, revealed little antigen in their walls, although antigen was in nearby tissues. This is in contradistinction to the classical Arthus phenomenon, which showed a predominantly vascular reaction and, using FA, localization of antigen within damaged vessel walls. Guinea pigs injected with BSA-rabbit and anti-BSA complexes showed rabbit globulin antibody distributed similarly to antigen. Intradermally injected soluble antigen-antibody complexes are capable of causing a cutaneous inflammatory reaction and necrotizing vasculitis without a demonstrable concentration of immunologic reactants in the vessel wall.

2470

Daems, W.Th.; Oort, J. 1962. Electron microscopic and histochemical observations on polymorphonuclear leukocytes in the reversed Arthus reaction. *Exp. Cell Res.* 28:11-20.

Granulocytes in the reversed Arthus reaction provoked with a bovine albumin antibovine albumin or a ferritin antiferritin system were studied with the electron microscope and by enzyme histochemistry. The cells showed large intracytoplasmic electron-dense lumps, consisting of antigen-antibody precipitate, as could be demonstrated in the ferritin antiferritin system. Furthermore, granules and mitochondria disappear from the cytoplasm, and the cells often show a vacuole situated underneath the nuclear membrane. These changes are discussed in relation to the findings in the literature. Part of the reaction is attributed to direct antigen-antibody interaction.

2480

Mellors, R.C.; Brzosko, W.J. 1962. Studies in molecular pathology: I. Localization and pathogenic role of heterologous immune complexes. *J. Exp. Med.* 115:891-902.

After intravenous injection into mice, rabbit immune complexes solubilized in antigen excess and containing fluorescent antigens or fluorescent antibody, or both, were localized in reticuloendothelial cells and polymorphonuclear leukocytes of the sinusoids of liver and the red pulp of spleen; in the kidney; in capillary endothelium of heart and lung; and in hepatic cells. Within 48 hours localization changed from the liver and splenic red pulp to the white pulp. At that time the kidney was cleared except for glomeruli. Other distributions and pathologic changes are discussed with reference to molecular weight of injected material and method of injection.

2490

Patterson, R.; Suszko, I.M.; Fruzansky, J.J. 1962. In vitro uptake of antigen-antibody complexes by phagocytic cells. *J. Immunol.* 89:471-482.

The ingestion of antigen-antibody complexes prepared as soluble materials was studied by a method of in vitro uptake by phagocytic cells using complexes labeled with iodine 131. Different degrees of in vitro pinocytosis occurred with preparations of complexes at different degrees of antigen excess. This in vitro pinocytosis was also demonstrated by a fluorescent antibody technique localizing the complexes intracellularly. These methods provide a system of study of transfer of soluble materials across the cell membranes of phagocytic cells that can be compared with the same phenomenon in vivo. The in vitro uptake of complexes was compared with antigen binding to globulin by the ammonium sulfate technique and the in vivo localization of radioactive trace-labeled antigen-antibody complexes. By these methods the ingestion of antigen-antibody complexes by cells may be studied in a simplified manner. The localization of antigen-antibody complexes in phagocytes occurs with complexes prepared with rabbit or human antiserum against serum protein antigens.

2500

Schlipkoter, H.W. 1961. Immunofluorescent localization of antigen-antibody complexes by examination with anti-lung serum. *Allergie Asthmaforsch* 4:308-310. In German.

In a fluorescent-serologic study of anti-lung sera one can, with the aid of labeled anti-antibody sera, demonstrate rabbit-albumin bodies in lung sections, in which the antibodies are localized. The demonstration of bound complement represents a valuable finding. One is thus able to visualize the places at which actual antigen-antibody reactions occur. However, since one demonstrates all complement-binding anti-antibody complexes, one also finds those that have no connection with the anti-lung serum. The studies of lungs of obviously healthy rats have shown that antigen-antibody reactions occur constantly in the lungs, called forth perhaps by inspired antigens.

2510

Vazquez, J.J.; Dixon, F.J.; Neil, A.L. 1957. Demonstration of specific antigen and antibody in experimentally produced amyloid. Amer. J. Pathol. 33:614.

Study with the fluorescent technique demonstrates that amyloid produced by the injection of casein contains specific antibody and antigen. Amyloid deposits in the spleen were produced in the rabbit by repeated subcutaneous injection of sodium caseinate. Specific rabbit anti-casein was obtained by immunization with the same material. The globulin fraction of this serum was labeled with fluorescein isocyanate and used to detect antigen and or antibody in the amyloid deposits. Such deposits contained appreciable concentrations of specific antibody, antigen casein, and to a lesser degree specific antigen. The localization of specific antibody corresponded with that of homologous gamma globulin. Parallel studies of similar amyloid deposits produced after repeated injections of ribose nucleic acid served as negative controls. Under the conditions of our experiment, amyloid deposits in the spleen of experimentally produced casein amyloidosis contain specific antibody and to a lesser degree antigen. Deposition of antigen and antibody may be one mechanism for the formation of amyloid.

2520

Weiser, R.S.; Laxson, C. 1962. The fate of fluorescein-labeled soluble antigen-antibody complex in the mouse. J. Infect. Dis. 111:55-58.

The use of fluorescein-tagged antibody for the preparation of soluble antigen-antibody complex was advantageous in tracing such complexes in the mouse. The fate of soluble antigen-antibody complexes composed of bovine serum albumin, BSA, and fluorescein-tagged anti-BSA of rabbit origin has been studied following intravenous injection in the mouse. Of the various organs and tissues studied, it was observed that the complex was initially deposited diffusely in the glomerulus and taken up by reticuloendothelial phagocytes of the liver and spleen. During the first few days, the complex disappeared from all sites except the glomerulus, where it accumulated to give dense focal deposits that commonly persisted for periods of one month or longer. Fluorescein-labeled anti-BSA did not persist in the glomeruli of control animals for more than a few days. The present results strengthen the concept expressed by Dixon and co-workers to account for the localization and prolonged persistence of antigen in the glomeruli of their actively sensitized rabbits, namely, that antigen combines with antibody to form a complex that is sequestered from phagocytic cells and proteolytic enzymes.

C. HYPERSENSITIVITY

2530

Cochrane, C.G. 1960. Applications of the fluorescent antibody technique to microbiology of the Arthus reaction. Ann. Inst. Pasteur 99:329-347. In French.

Studies of the Arthus phenomenon indicated that the relative severity of active Arthus and reversed passive Arthus reactions, in contrast to the more mild local passive Arthus and the reaction to locally injected soluble antigen-antibody complexes, is due to a concentration of antigen and globulin, without doubt antibody, in the vessels of the former two reactions. This would bring about selective destruction of the vessels, allowing the severe reactions to follow. The polymorphonuclear leukocyte is essential for the development of the Arthus-type vasculitis. Antigen and globulin were found deposited in vessel walls but in the absence of polymorphs no visible reaction occurred in these vessels. It was also found that leukocytes, especially polymorphs, are responsible for removing some of the antigen from the sites of deposition in Arthus reactions. This occurs in part through direct catabolism of the antigen, completed with globulin. Healing of such reactions is dependent upon the removal of the antigen-antibody complex.

2540

Cochrane, C.G.; Weigle, W.O. 1958. The cutaneous reaction to soluble antigen-antibody complexes: A comparison with the Arthus phenomenon. J. Exp. Med. 108:591-604.

The *in vivo* activity of soluble antigen-antibody complexes was tested by a single intradermal injection in rabbits. Skin reactions were marked by erythema, induration, and occasionally hemorrhage and necrosis. Microscopically, diffuse inflammation and occasional vascular necrosis could be found. This indicates that soluble antigen-antibody complexes are phlogogenic and are responsible for serum sickness. The reactions were similar in severity to local passive Arthus, LPA, reactions at equal dosages of antibody. BSA antigen could be found in large concentrations in affected vessel walls of both reverse passive Arthus, RPA, and active or classical Arthus reactions. This vascular localization of antigen might bring about the relative severity of the RPA and active Arthus reactions, as contrasted to the complex and LPA reactions. The finding of affected vessels in the complex and LPA reactions containing little or no antigen and antibody, although they were present in adjacent areas, suggests that antigen-antibody combination may cause vascular reactions and damage by release of physiologically active mediators from tissue or tissue fluid.

2550

Cochrane, C.G.; Weigle, W.O.; Dixon, F.J. 1959. Factors responsible for decline of inflammation in Arthus hypersensitivity vasculitis. Proc. Soc. Exp. Biol. Med. 101:695-699.

Studies suggest that neither exhaustion of humoral or cellular factors necessary for inflammation nor presence of possible inhibitors of the reaction play a dominant role in diminution of inflammation and healing of a hypersensitivity vasculitis of the Arthus type. This study supports the importance of ridding the diseased vessel of antigen and antibody in bringing about a decrease in reaction and healing.

2560

Fisher, E.R.; Fisher, B. 1959. Investigation of rejection of canine renal homotransplants by fluorescent antibody technic. Proc. Soc. Exp. Biol. Med. 101:259-261.

No preferential localization of globulin was noted in rejected homotransplanted canine kidneys studied by fluorescent antibody technique. Indirect variation of this method similarly failed to disclose antibodies exhibiting an affinity for renal structure in serum of recipients exhibiting rejection of renal homotransplants. The fibrin nature of fibrinoid lesions occasionally noted in terminal phases of the rejection phenomenon has been demonstrated. Relationship of pathologic and immunohistochemical alterations of renal homotransplantation rejection and the generalized Shwartzman phenomenon are discussed.

2570

Germuth, F.G.; Maumenee, A.E.; Senterfit, L.B.; Pollack, A.D. 1962. Immunohistologic studies on antigen antibody reactions in the avascular cornea: I. Reactions in rabbits actively sensitized to foreign protein. J. Exp. Med. 115:919-928.

The injection of antigen into the center of the avascular cornea of homologously sensitized animals induced a ring of opacification composed of a line of deeply eosinophilic amorphous material in a matrix of swollen collagen fibers, palisaded by polymorphonuclear leukocytes. By use of fluorescein-tagged antigen, it was shown that the line of damage in the cornea coincided with the precipitation of antigen, presumably by antibody entering the cornea from the limbal vessels. With the passage of time, the antigen-antibody precipitates were removed, at least in part by phagocytosis, and the ring of opacification was replaced by ingrowing blood vessels surrounded by plasmal cells. Treatment of sensitized animals with nitrogen mustard showed that antigen-antibody interaction could injure the corneal stroma in the relative absence of polymorphonuclear leukocytes.

2580

Kyogoku, M. 1962. A study on Arthus phenomenon using fluorescein-labeled protein. Acta Sch. Med. Univ. Kioto 38:71-100.

A single-step fluorescent antigen method was used to detect anti-ovalbumin antibody in rabbits. Day-by-day distribution of antigen and antibody is described. On injection, part of the antigen reacts with antibody on blood vessel walls, while another granular part disperses to tissue spaces. Some antigen reacts with collagen fiber bundles. Most of the antigen is inactive by the 4th day and is formed as a crust in demarcated, necrotized tissues. Some is phagocytized by macrophages and is active until the 6th day. Fibrinoid swelling results from invasion of antibody into the separated microfibrils. Antibody in cutis increases suddenly on the 3rd or 4th day. Local antibody is produced on the 5th or 6th day. Plasma cells in cutis produce antibody that is given off as granules that dissolve. Russell bodies were not seen. Histiocytes contain antibody on the 3rd day, but granulocytes, lymphocytes, erythrocytes, and mast cells never contain antigen or antibody. Antibody is still abundant in plasma cells on day 14.

2590

McKay, D.G.; Gitlin, D.; Craig, J.M. 1959. Immunochemical demonstration of fibrin in the generalized Shwartzman reaction. AMA Arch. Pathol. 67:270-273.

Use of the fluorescent-antibody technique has demonstrated that fibrin or an insoluble derivative of fibrinogen is a constituent of the thrombi found in the lungs, spleen, liver, and kidneys in the generalized Shwartzman reaction. In combination with studies of the blood coagulation mechanism, it is concluded that fibrin is the essential constituent of these thrombi.

2600

McKinnon, G.E.; Andrews, E.C.; Heptinstall, R.H.; Germuth, F.G. 1957. An immunohistologic study on the occurrence of intravascular antigen-antibody precipitation and its role in anaphylaxis in the rabbit. Bull. Johns Hopkins Hosp. 100:258-280.

Large amounts of antigen and antibody are required for the induction of anaphylactic shock in rabbits. In vivo intravascular precipitation of antigen with antibody, as demonstrated by the use of fluorescein-labeled antigen, regularly occurs under these conditions. Obstruction of the pulmonary capillaries by immune precipitates appears to be an important mechanism in the pathogenesis of anaphylactic shock in the rabbit. Obstruction of the portal veins and sinusoids of the liver by immune precipitates may cause infarction of the portal and midzonal areas of the liver lobule, especially when the challenging antigen is given via the portal circulation. The foregoing facts suggest an explanation for the unusual aspects of anaphylaxis in the rabbit and must be considered when comparisons are made with other animals or man where in vivo antigen-antibody precipitation has not been demonstrated.

2610

Oort, J.; van Rijssel, Th.G. 1961. Fluorescent protein tracer studies in allergic reactions: I. The fate of fluorescent antigen in active and passive Arthus reactions in guinea pig skin. Immunology 4:329-336.

The fate of intradermally injected bovine serum labeled with lissamine rhodamine was studied in actively and passively sensitized guinea pigs and compared with the fate of the same protein in control animals. In the latter, the antigen was taken up by macrophages, apart from a discharge of antigen to the regional lymph nodes. In the sensitized animals, in which severe Arthus reactions occurred, antigen accumulated in vessel walls and lumina of capillaries and venules. Polynuclear leukocytes take antigen after a few hours. This is a specific reaction absent with simultaneously injected protein to which the animal is not sensitized.

2620

Rappaport, B.Z. 1960. Antigen-antibody reactions in allergic human tissue: I. Preparation and use of fluorescein-conjugated reagins for staining the reaction site. *J. Exp. Med.* 112:55-64.

Skin-sensitizing human antibodies were conjugated with various fluorescent dyes without significant loss in their ability to combine with specific antigen in vitro. A biopsy of the skin site challenged with egg albumin in a patient sensitive to this antigen could be stained specifically by FA. Epithelial cells of the epidermis, sweat glands, hair follicles, and sebaceous glands in such a challenged site showed specific staining. In addition to the epithelial cells, the most intense staining was in macrophages and in pericapillary cells. The endothelium of the small blood vessels stained less intensely. Fibrous tissue bundles were specifically stained. The immunologic staining with the conjugated reagins was similar to but more intense than that obtained with conjugated rabbit anti-egg albumin globulins.

2630

Rappaport, B.Z. 1960. Antigen-antibody reactions in allergic human tissue: II. Study by fluorescence technique of the localization of reagins in human skin and their relation to globulins. *J. Exp. Med.* 112:725-734.

The skin of atopic patients contains specific reaginic antibodies in all epidermal cells, in unchallenged as well as antigen-challenged areas. The reagins are also present in the epithelial cells of sebaceous glands, sweat glands, hair follicles, and in macrophages and pericytes. Judged by staining characteristics, their immunologic reaction in tissues with antigen is identical to that of rabbit antibodies. Comparison of tissues stained with conjugated reagins and conjugated antihuman globulin antibodies demonstrated the close relationship of reagins and globulins. In antigen-challenged tissues the macrophages and pericytes become enlarged and stain more intensely with toluidine blue. No such changes in morphology or staining are present in histamine-tested or unchallenged atopic tissues.

2640

Raskin, J. 1961. Antigen antibody reaction site in contact dermatitis: Determination by use of fluorescent antibody technique. *Arch. Dermatol.* 83:459-465.

Fluorescent antibody was used to identify the site of the antigen-antibody reaction occurring in Rhus dermatitis experimentally produced in ten human subjects. That the upper dermis, especially the papillary region, was principally involved was supported by the constant position of the yellow-green fluorescence, the quantitative correlation with severity of the cutaneous reaction, absence of specific fluorescence in control specimens, and the results of the tests for specificity. The reaction did not involve the epidermis, even in areas in which the dermatitis had been present for as long as 48 hours. Attempts to determine the site of the antigen-antibody reaction in subjects sensitized to 2,4-dinitro-1-chlorobenzene were unsuccessful. Reasons for the failure are discussed.

2650

Vainio, T.; Saxen, L.; Toivonen, S.; Rapola, J. 1962. The transmission problem in primary embryonic induction. *Exp. Cell Res.* 27:527-538.

Fluorescent antisera against a heterogenous inductor tissue was used to detect and localize transferred antigenic material in the ectoderm. After 3 to 6 hours of contact between the tissues, antigenic granules were obtained in abundance inside the ectodermal cells. The results demonstrate a transfer of large-molecule antigenic material during the induction process, and show that the inductive information could be transmitted through a similar mechanism. Whether this is in fact the case cannot yet be concluded.

2660

van den Berg, C.; Oort, J.; van Rijssel, Th.G. 1962. Fluorescent protein tracer studies in allergic reactions: II. The fate of fluorescent antigen in reversed Arthus reactions and passive cutaneous anaphylaxis in guinea pig skin. *Immunology* 5:389-393.

The fate of lissamine rhodamine-labeled bovine serum albumin was studied in reversed Arthus reactions and passive cutaneous anaphylaxis, PCA. In reversed Arthus reactions antigen accumulated in the subendothelial spaces and in the endothelial cell layer of vessel walls and in the lumina of capillaries and venules as well as extracellularly. After some time granulocytes took up antigen. In PCA the deposits in vessel walls were not impressive but clearly demonstrable; granulocytes take up antigen within 5 minutes.

2670

Witmer, R.H. 1955. Antibody formation in rabbit eye studies with fluorescein-labeled antibody. *AMA Arch. Ophthalmol.* 53:811-816.

Local antibody formation takes place in the inflamed eye after injection of egg albumen into the vitreous and into the anterior chamber. By use of fluorescein-labeled antibody combined with pretreatment of the frozen sections of uveal tissue with a dilute antigen solution, it was found that the cells containing antibody are plasma cells. There was a rough correlation between the antibody activity of the gamma globulins of the aqueous humor - determined by means of hemagglutination and paper electrophoresis - and the number of antibody-containing plasma cells in the uveal tissue. Caterpillar hair in the anterior chamber as a local irritant seemed to act as a slightly enhancing factor in antibody production, and antibody-containing cells were found in the granulomata around the hair.

2680

Wittmer, V.R. 1955. Antibody formation in the eye. *Schweiz. Med. Wochensch.* 85:332-333. In German.

The Coons technique of fluorescein-labeled antibody makes it possible to demonstrate local antibody formation in the inflamed eye. It was found that plasma cells infiltrating the uveal tissue contained antibody, although the mesenchymal tissue of the iris and ciliary body did not.

VI. NATIVE ORGAN AND TISSUE ANTIGENS

A. ORIGIN AND DISTRIBUTION

2690

Barnhart, M.I.; Anderson, G.F. 1960. Demonstration of prothrombin in liver parenchymal cells using fluorescent antibody. Federation Proc. 19:58.

Previous studies implicate the liver but have not identified the cell type responsible for synthesis of any one of the tract plasma proteins concerned in blood coagulation. In this study to find out the cellular site for prothrombin synthesis, fluorescent antibody and other immunochemical procedures were utilized. With dog prothrombin as the antigen, high-titered antiprothrombin sera were produced in rabbits. Dog prothrombin mixed with aluminum hydroxide and injected intramuscularly gave potent antisera. Globulin concentrates were coupled with rhodamine and applied to dog liver sections or imprints. Such antiprothrombins precipitated on parenchymal cells containing sufficient prothrombin. Absorption of antiprothrombin by dog prothrombin reduced the specific orange fluorescence. Uncoupled antiprothrombin serum suppressed fluorescence. Autofluorescence varied with the fixatives. It appears that antiprothrombin is specific in its reaction with prothrombin of the liver parenchymal cell. However, liver parenchymal cells did not react uniformly with antibody. This may mean that a certain type of liver parenchymal cell produces prothrombin or that there is cyclic production of prothrombin.

2700

Barnhart, M.I.; Anderson, G.F. 1962. Cellular study of drug alteration of prothrombin synthesis. Biochem. Pharmacol. 9:23-27.

Anti-dog prothrombin was conjugated and used to identify cells containing prothrombin in dog liver imprints. Liver cell fluorescence and blood prothrombin levels were compared following treatment with coumarin drugs and vitamin K1. Liver parenchymal cells, not reticuloendothelial cells, normally synthesize prothrombin.

2710

Baum, J. 1959. Reaction of guinea pig spermatozoa with homologous antibody as demonstrated by fluorescent staining. Lancet 1:810.

Antisera prepared against guinea pig sperm or homologous testis and subsequently conjugated with fluorescein stains selectively the heads of the mature spermatozoa in sections. It is postulated that the antibody thus prepared is against protamine, the end substance in the sperm maturation process.

2720

Baum, J.; Boughton, B.; Mongar, J.L.; Schild, H.O. 1961. Autosensitization by sperm in guinea pigs. Immunology 4:95-110.

Guinea pigs injected intradermally with homologous sperm or testis in Freund adjuvant develop various manifestations of hypersensitivity. Living sperm was antigenic but even

more so after freezing and thawing. Sperm antigen is stable at 56°C but is inactivated in direct proportion to increasing time at 100°C. Transmittance of hypersensitivity by implantation of lymph node cells into skin of normal animals was not successful. Fixation of antibody on the acrosomal portions of sperm was demonstrated by fluorescent anti-globulin serum. The antigenic component of sperm may be mucopolysaccharide.

2730

Beck, J.S.; Edwards, R.G.; Young, M.R. 1962. Immune fluorescence technique and the isoantigenicity of mammalian spermatozoa. *J. Reprod. Fertil.* 4:105-110.

FA has been used to study reactions between serum and spermatozoa from several species of mammals. Normal serum from adult guinea pigs, rabbits, mice, and men stained the acrosomes of homologous and heterologous spermatozoa, staining being most intense with rabbit and guinea pig spermatozoa. However, staining was weak with sera from rabbits up to 3 weeks of age, and could be removed by heating or Seitz-filtering the sera. Sera from immature guinea pigs likewise lost most of their staining capacity on heating. Differences in the intensity of staining appeared to be determined by the species of spermatozoa rather than by the species of serum. Serum from guinea pigs immunized with homologous spermatozoa also stained the surface of homologous sperm tails. A slight cross-reaction occurred between this serum and the tails of hamster spermatozoa, but not with spermatozoa from other species. Immunization of rabbits with homologous spermatozoa failed to produce any tail staining. The two reactions between serum and spermatozoa, i.e. with acrosomes and tails, are discussed in relation to the isoantigenicity of spermatozoa and to induced aspermatogenesis following the injection of testis or spermatozoa and adjuvant.

2740

Cruickshank, B.; Hill, A.G.S. 1953. The histochemical identification of a connective tissue antigen in the rat. *J. Pathol. Bacteriol.* 66:283-289.

Globulin from an antiserum prepared against whole rat kidney and conjugated with a fluorescein derivative has been shown by fluorescence microscopy to react with basement membrane and reticulum in many rat organs and with sarcolemma and neurilemma. A more limited study has shown that anti-rat glomerulus and anti-rat lung globulins react similarly. The nature of the antigenic substance with which the globulins react has not been determined, but it is possibly a mucopolysaccharide. Globulins that react with reticulum do not react with collagen.

2750

Ehrenpreis, S. 1962. Immunohistochemical localization of drug-binding protein in tissues of the electric eel. *Nature* 194:586-587.

A protein with a high affinity for d-tubocurarine had been isolated from ammonium sulfate fractions of extracts of electric organs of the eel. One method for rather precise localization of the protein is the fluorescent antibody technique, the results of which are reported here.

2760

Emmart, E.W.; Spicer, S.S. 1961. The localization of glyceraldehyde-3-phosphate dehydrogenase in roach muscle by staining with fluorescent antibody. Federation Proc. 20:16.

Recent studies on fractions of tissue homogenates have demonstrated the presence of glyceraldehyde-3-phosphate dehydrogenase, GAPD, in the thoracic and leg muscles of insects. Fluorescent antibody has been used to localize the enzyme. Guinea pigs have been injected with GAPD isolated from rabbit muscle and five times recrystallized. The course of antibody development was followed by increase in per cent protein of the gamma globulin and by precipitin reaction in solution and in agar. The enzyme has been identified and localized in the mitochondria of the freshly teased muscle fibers of the wing and leg muscles by binding the enzyme with specific fluorescent antibody solution. GAPD is intimately associated with the mitochondria of the isotropic or I band. The large mitochondria dispersed linearly between the myofibrils also stain positively but with less intensity. The localization of these mitochondria in relation to the I and A bands of the myofibril has been determined by histochemical staining as well as by staining the myofibril with fluorescent antimyosin globulin.

2770

Emmart, E.W.; Spicer, S.S.; Turner, W.A.; Henson, J.G. 1962. The localization of glyceraldehyde-3-phosphate dehydrogenase within the muscle of the roach, Periplaneta americana, by means of fluorescent antibody. Exp. Cell Res. 26:78-97.

Precipitating antiserum has been obtained from guinea pigs inoculated subcutaneously with glyceraldehyde-3-phosphate dehydrogenase prepared from rabbit muscle and five times recrystallized. Pooled globulin fractions of immune guinea pig sera conjugated to fluorescein isocyanate were used to demonstrate the enzyme in mitochondria of freshly teased, unfixed fibers of wing and leg muscles of the cockroach. The enzyme was confined to the mitochondria and was not in the sarcoplasm. Comparison of fibers stained by FA and histochemical staining techniques demonstrated that mitochondria containing GAPD encircle myofibrils peripheral to the I band and give a cross-striated appearance to the resting fiber. The enzyme is not species-specific.

2780

Finck, H.; Holtzer, H. 1961. Attempts to detect myosin and actin in cilia and flagella. Exp. Cell Res. 23:251-257.

Antisera against chicken skeletal muscle myosin and actin were used to test for the presence of these proteins in chicken ciliated epithelium and sperm. The fluorescent antibody method and precipitin reactions in gels both showed that there are no antigenic similarities between the contractile proteins of skeletal muscle and those of cilia and flagella.

2790

Finck, H.; Holtzer, H.; Marshall, J.M. 1956. An immunochemical study of the distribution of myosin in glycerol-extracted muscle. *J. Biophys. Biochem. Cytol.* 2:Part 2:175-178.

Recent investigations of the distribution of myosin and actin in the skeletal muscle fiber have relied largely on extraction techniques for the differential removal of specific proteins. This work has been reviewed by Hanson and Huxley. Because such extraction techniques may remove much soluble material in addition to myosin or actin, conclusions based on density changes within fibers after extractions have seemed somewhat questionable. We have, therefore, attempted a more positive localization of myosin in glycerol-extracted fibers by the fluorescent antibody method.

2800

Nelander, E.; Emmart, E.W. 1959. Localization of myosin in the conduction bundle of beef heart. *Proc. Soc. Exp. Biol. Med.* 101:838-842.

Positive binding of fluorescent antimyosin solution to myofibrils of the Purkinje cell demonstrates presence of myosin in myofibrils of the conduction bundle of beef heart. Antibody to myosin, whether prepared from skeletal muscle or heart tissue, is capable of binding heart muscle antigen.

2810

Hill, A.G.S.; Cruickshank, B. 1953. A study of antigenic components of kidney tissue. *Brit. J. Exp. Pathol.* 34:27-34.

The properties of antibodies prepared against homogenates of whole kidney, isolated glomeruli, and lung from normal rats have been investigated. Fluorescein-labeled globulin from each of these antisera has been applied to unfixed sections of normal rat kidney and the sites of specific fixation have been determined by fluorescence microscopy.

2820

Hiramoto, R.; Calcagno, P.; Pressman, D. 1952. Localization of anti-rat kidney antibodies in newborn rats. *Proc. Soc. Exp. Biol. Med.* 110:482-485.

Rabbit antibodies against adult rat kidney were found to be fixed in the kidneys of newborn rats. Since newborn rats do not develop clinical disease when injected with anti-rat kidney antibodies this suggests either that the antigens giving rise to cytotoxic antibodies are not present at this early date or that the newborn rats are deficient in complement. Six weeks after newborn rats are so injected they still have the rabbit globulin antibody in the glomeruli. However, rabbit globulin was demonstrated in the glomeruli on the medullary side of the cortex and not in those of the periphery, indicating that new glomeruli become functional as the animal matures. The increase in functional glomeruli may be the reason for the observation that newborn rats injected with rat kidney antiserum do not develop clinical disease.

2830

Hiramoto, R.; Jurandowski, J.; Bernecky, J.; Pressman, D. 1959. Precise zone of localization of anti-kidney antibody in various organs. Proc. Soc. Exp. Biol. Med. 101: 583-586.

In vivo localization of antibodies prepared in rabbits against rat kidney was determined by immunohistochemical procedures. Fixation to high concentration was observed in certain structures of ovary and spleen as well as in kidney and adrenal. A lower concentration was observed on structures in thyroid, lymph node, and liver but none was seen in testes, lung, skin, brain, and heart. Localization of these antibodies in vivo is quite different from that observed by treating tissue sections in vitro with the antibody. In the latter case, many more structures are stained.

2840

Hiramoto, R.; Yagi, Y.; Pressman, D. 1958. Adrenal localization of anti-rat kidney antibodies. Federation Proc. 17:2017:516.

When the globulin fraction of rabbit antiserum prepared against rat kidney, G-anti-RK, is injected into rats, antibody is localized in the adrenals and kidney. These experiments with fluorescent antibody determine the histologic site of localization. G-anti-RK and globulin fraction of normal serum were injected intravenously into rats, and the animals were sacrificed after 24 hours and perfused to remove excess rabbit globulin. Frozen sections of the kidneys and adrenals were stained with fluorescein or tetramethylrhodamine-labeled horse and rabbit globulin. Localization of rabbit globulin was found only in the case of animals injected with G-anti-RK and occurred primarily in the lining of the sinusoids and vessels of the adrenal gland and not in the capsule or the gland cells. When sections of the adrenal from uninjected rats were treated with G-anti-RK and subsequently stained, there was staining of the capsule in vitro as well as the sinusoidal elements of the gland. Comparison of the radio and fluorescent label methods showed that the amount of globulin required for detection by the fluorescein technique was orders of magnitude greater than that required by the radio technique. The fluorescein label gives better definition of localization, but the radio label permits localization of smaller quantities of globulin.

2850

Hiramoto, R.; Yagi, Y.; Pressman, D. 1958. In vivo fixation of antibodies in the adrenal. Proc. Soc. Exp. Biol. Med. 98:870-874.

The site of fixation in the adrenal gland of rat kidney antibody administered in vivo has been demonstrated by the immunohistochemical technique to be along the vessels and sinusoids of the cortex. There is no fixation in the medulla, although the medulla does contain antigen capable of reacting with the antibody in vitro. The possible derangement of adrenal function may be due to the effect of the anti-kidney antibodies.

2860

Holtzer, H. 1959. Some further uses of antibodies for analyzing the structure and development of muscle. *Exp. Cell Res. Suppl.* 7:234-243.

Preliminary results using labeled and unlabeled muscle antibodies to follow the distribution of muscle proteins in normal and extracted myofibrils are reported. Application of immunological methods to the analysis of differentiating embryonic cells and, more specifically, to the differentiating muscle cell is discussed. Until embryologists are at least as critical of their immunological techniques as the immunologists are, their findings must be viewed with scepticism. The claim that heart antigens, spleen antigens, or nerve antigens are to be found in the early chick blastoderm is based on the cytotoxic effects of crude antisera. Until more substantial evidence is forthcoming on this point such claims cannot be considered proven. Furthermore, in our experience antisera to reasonably defined intracellular muscle antigens are not selectively cytotoxic to living muscle, are not adsorbed on the surface of living muscle, and are not able to enter living muscle.

2870

Holtzer, H.; Abbott, J.; Cavanaugh, N.W. 1959. Some properties of embryonic cardiac myoblasts. *Exp. Cell Res.* 16:595-601.

Under *in vitro* conditions, FA staining with antimyosin conjugate reveals myofibrils at a development stage when cardiac myoblasts normally appear to lack them. By using labeled antimyosin, typically striated fibrils are found in large numbers of myoblasts before the heart starts to beat in the chick embryo. The localization of fluorescein-labeled antimyosin along the myofibrils of cardiac and skeletal myoblasts is similar to its distribution along the myofibrils of mature cardiac and skeletal muscle. Similarity of cardiac and skeletal myosins has been demonstrated by the capacity of glycerinated cardiac myofibrils to absorb skeletal antimyosin antibodies. The insoluble fibrous protein of glycerol-extracted mitotic figures fails to bind fluorescein-labeled antimyosin.

2880

Holtzer, H.; Marshall, J.M.; Finck, H. 1957. An analysis of myogenesis by the use of fluorescent antimyosin. *J. Biophys. Biochem. Cytol.* 3:705-724.

Antibodies against myosin of adult chicken skeletal muscle were labeled with fluorescein and used as staining reagents to analyze the development of trunk myoblasts in the chick embryo. Myoblasts from the brachial myotomes were studied in three ways: Specimens were fixed, sectioned, and stained with iron hematoxylin. Living myoblasts, and myoblasts prepared by glycerol extraction, were teased and examined by phase contrast microscopy. Embryo trunks were treated with fluorescent antimyosin or with a control solution of fluorescent normal globulin and were examined by fluorescence and phase contrast microscopy. Both glycerol-extracted and fixed materials were used.

2890

Kaplan, M.H. 1958. Immunologic studies of heart tissue: II. Differentiation of a myocardial sarcoplasmic antigen and cardiolipin. J. Immunol. 30:268-277.

An alcohol-extractable sarcoplasmic antigen previously identified in the hearts of several mammalian species may be differentiated from cardiolipin by absorption and immunohistochemical tests. Antibody titers to these two antigens varied independently in era of rabbits immunized with homogenates of beef heart or rat heart tissue. Absorption of these sera with cardiolipin did not remove antibody to the sarcoplasmic antigen, but absorption with heart tissue homogenate removed both antibodies. Both antigens are constituents of the myocardial cell sarcoplasm; however, differences in their histochemical properties can be demonstrated.

2900

Klatzo, I.; Horvath, B.; Emmart, E.W. 1958. Demonstration of myosin in human striated muscle by fluorescent antibody. Proc. Soc. Exp. Biol. Med. 97:135-140.

Fluorescent antibody was used to study the localization of myosin in normal and diseased skeletal muscle. A method for assay of antibody to human myosin is described. Specific staining with fluorescent antibody was obtained in A bands of the striated muscle. According to observations on staining with fluorescent antibody and to precipitin reactions, myosin must be considered not species-specific. Preliminary observations on diseased muscle stained with fluorescent antibody revealed the preservation of striated pattern at the time of early pathology changes and the presence of material reacting with the antibody to human myosin in severely degenerated fibers and in structures resembling macrophages.

2910

Mancini, R.E.; Davison, O.W.; Vilar, O.; Nemirovsky, M.; Bueno, D.C. 1962. Localization of achrosomal antigenicity in guinea pig testes. Proc. Soc. Exp. Biol. Med. 111: 435-438.

Morphologic damage of the adult guinea pig testis can be induced by the single intracutaneous injection of homologous testes extract plus complete Freund adjuvants. The characteristic feature of this phenomenon is sloughing of the germinal epithelium so extensive as to result in complete aspermatogenesis. Vacuolization of spermatogonia, pycnosis of germ cell nuclei, appearance of multinucleated cells, and cytoplasmic vacuolization of Sertoli cells have been related morphologic findings; no influence on the Leydig cells has been observed. That this reaction is due to an immunologic mechanism has been amply demonstrated. Various authors have reported the presence of circulating antibodies, delayed cutaneous hypersensitivity, and effectiveness of passive transfer by white cells from sensitized donors. Attempts have been made to characterize chemically the antigen responsible for the induced aspermatogenesis. Since the initial work of Freund, attention has been focused upon the carbohydrate-rich achromosome and it has been known that polysaccharide fractions of testicular extracts are active. Other sperm antigens have been identified, including hyaluronidase, a substance containing nucleic acid, and at least one unidentified protein.

2920

Marshall, J.M.; Holtzer, H.; Finck, H.; Pepe, F. 1959. The distribution of protein antigens in striated myofibrils. *Exp. Cell Res. Suppl.* 7:219-233.

The method of localizing specific protein antigens by antibody staining has been applied to skeletal muscle. Some progress has been made in determining the distributions of myosin, actin, and tropomyosin within the sarcomere. The fundamental problem of determining the immunochemical specificity of such staining procedures has been considered. This has led to new studies on the splitting of myosin by trypsin, on the properties of the four major fragments derived from myosin by brief digestion, and on the differential localization of these parts of myosin within the A band region of the sarcomere. The L-1 portion of myosin lies in the lateral edges of the A band of the relaxed sarcomere. The other parts of the myosin complex appear to lie in the more central region of the A band, but detailed mapping and immunochemical identification of these parts remains to be done. It is suggested that the myosin molecule in its native state may be greatly extended.

2930

Mellors, R.C.; Siegel, M.; Pressman, D. 1955. Analytic pathology: I. Histochemical demonstration of antibody localization in tissues with special reference to the antigenic components of kidney and lung. *Lab. Invest.* 4:69-89.

Detailed instructions are presented for the *in vivo* localization of antigens by FA. The kidney and lung were studied, and problems encountered in estimating degree of fluorescence in lung sections and proper interpretation are discussed. Antibodies against kidney and lung localize in the kidney. The important antigens in the kidney are the basement membranes of the glomeruli. Antibodies localize in the glomeruli during acute experimental glomerulonephritis in rabbits.

2940

Moeller, G. 1961. Demonstration of mouse isoantigens at the cellular level by the fluorescent antibody technique. *J. Exp. Med.* 114:415-433.

FA has been used to demonstrate mouse isoantigens at the cellular level. Specific reactions were obtained by the indirect or sandwich technique with a variety of living normal and neoplastic cells. Isoantigens of the H-2 system and of other systems could be demonstrated as well and appeared to be localized at the cell membrane. In the H-2 system, membrane localization could be confirmed histologically. Different types of nonspecific staining reactions have been identified and described. Pinocytosis and cell injury leading to reactions that were morphologically indistinguishable from the specific ring reaction as far as pinocytosis is concerned, could be easily avoided by reducing the incubation time. A nonspecific staining reaction morphologically indistinguishable from the specific ring reaction could be seen in a small proportion of bone marrow and lymph node cells but in no other cell type. The possible nature of this reaction is discussed.

2950

Nace, G.W.; Clarke, W.M. 1958. Immunohistological localization of one or more transitory antigens in the optic cup and lens of Rana pipiens, p. 546-561. In W.D. McElroy and B. Glass, ed. The chemical basis of development. The Johns Hopkins Press, Baltimore.

The globulin fractions of antisera directed against supernatants of the tail bud of stages 19 and 20 of the frog Rana pipiens were conjugated with fluorescein isocyanate. Such reagents were compared with antisera against mature ovarian oocyte supernatants in interface precipitin reactions and Ouchterlony agar diffusion. Selected conjugates were applied to frozen and paraffin-embedded embryo sections, which were examined for specific localization of the antibody. Among others, localization was observed in the optic cup and lens of stage 19 and 20 embryos, but not in the optic vesicle or presumptive lens ectoderm of stage 17 or in any of the eye structures of stage 21. Evidence suggests localization of this reaction in the nuclei and cytoplasm of the optic cup, but only in the cytoplasm of the primordial lens. The possible nature of this transitory antigen and its relation to lens differentiation are discussed.

2960

Nairn, R.C.; Ghose, T.; Fothergill, J.E.; McEntegart, M.G. 1962. Kidney-specific antigen and its species distribution. Nature 196:385-387.

Kidney-specific antisera were conjugated and used in homologous and heterologous staining among various species. Variations of staining were noted. Kidney-specific material was particularly abundant in fresh-water fish.

2970

Roberts, D.St.C. 1957. Studies of the antigenic structure of the eye using the fluorescent antibody technique. Brit. J. Ophthalmol. 41:338-347.

The application of the fluorescent antibody-antigen technique in ophthalmic pathology is discussed. An antiserum to rat glomerular basement membrane was produced and was shown to react specifically with membranes in the ciliary body and iris and also with membranes in the small blood vessels of the retina, optic nerve, and conjunctiva. The lens capsule shared this antigenicity and seems to resemble basement membranes elsewhere in the body.

2980

Sargent, A.U.; Richter, M.; Medzon, J.; Rose, B. 1962. Organ and species specificity of rat liver antigen. Federation Proc. 21:20.

Organ and species specificity of rat liver antigen was studied by complement fixation, tanned red cell agglutination, and indirect fluorescein staining technique. A potent rabbit antirat liver serum was obtained against rat liver homogenate rendered free of connective tissue and vascular elements. Rabbit antiserum against rat heart, lung, and kidney was also prepared. Organ specificity was shown by a failure of rabbit antirat liver sera to react directly with the aforementioned rat organ antigens. The rabbit antisera against these same organ antigens also failed to react when tested directly against rat liver antigen. Species specificity was indicated by a failure to inhibit

the rat liver rabbit antirat liver system by prior incubation of the antisera with saline extracts of heterologous liver antigens. The finding of both organ- and species-specific antigen in the liver strengthens the possibility that a true autoimmune disease involving the liver can occur.

2990

Sarre, H.; Rother, K. 1957. Antibodies directed against the kidney, p. 95-133. In P. Miescher and K.O. Vorlaender, ed. Die immunopathologie in klinik und forschung und das problem der autoantikörper. George Thieme Verlag, Stuttgart.

Fluorescent antibodies were used to localize cytotoxic antibodies in the kidney. The antibody of isolated globulin fractions of antiserum to mouse kidney or rat kidney was bound in the kidney of mice or rats, mainly in the glomerulus.

3000

Stockdale, F.E.; Holtzer, H. 1961. DNA synthesis and myogenesis. *Exp. Cell Res.* 24: 508-520.

Differentiated muscle cells synthesizing myosin, the meromyosins, and actin do not concurrently synthesize DNA. Presumptive myoblasts that synthesize DNA do not concurrently synthesize myosin, the meromyosins, or actin. The multinucleated muscle fiber is the product of cell fusion.

3010

Tamanai, I.; Yagi, Y.; Hiramoto, R.; Pressman, D. 1961. Lung-localizing antibodies in anti-lung and anti-kidney serum. *Proc. Soc. Exp. Biol. Med.* 106:661-663.

Localization of antibodies in lungs was examined by fluorescent antibody after injection of rabbit antisera to rat lung and rat kidney into rats. Although both sera gave positive staining in lungs, anti-lung serum contained significantly higher amounts of lung-localizing antibodies than anti-kidney serum. The localizing components in both sera seemed to be fixed in the same region of alveolar walls.

3020

Tunik, B.; Holtzer, H. 1961. The distribution of muscle antigens in contracted myofibrils determined by fluorescein-labeled antibodies. *J. Biophys. Biochem. Cytol.* 11:67-75.

Chick myofibrils in different states of contraction were treated with fluorescein-labeled antibodies. The rabbit antibodies were prepared against chick myosin, light and heavy meromyosins, and actin. For any one state of contraction a single myofibril was photographed through the phase contrast microscope, stained with one of the antisera, and photographed through the fluorescence microscope. The cytological changes in the sarcomeres accompanying contraction, as observed under the phase contrast microscope, were correlated with changes in the distribution of the precipitated antibodies as observed under the fluorescence microscope. The changing patterns observed through the fluorescence microscope were compared with those predicted by the sliding filament model of contraction.

3030

Weil, A.J.; Rodenburg, J.M. 1962. The spermatozoa-coating antigen of the seminal plasma. Federation Proc. 21:44.

Seminal plasma of man and other mammals contains a strongly antigenic, species- and organ-specific material. It forms a firmly adherent coat on the surface of the spermatozoa during their passage through the adnexal structures of the male genital tract. This coat dominates the immunological behavior of seminal spermatozoa. The spermatozoa-coating antigen, SCA, is isoantigenic in the rabbit. It can be visibly demonstrated on the spermatozoa with fluorescein-conjugated anti-SCA immune globulin. This reagent specifically stains small granules in the epithelial cells lining the lumen and in those of the intramural glands of rabbit seminal vesicles; it is also present within the lumen. It appears, therefore, that the SCA originates in the seminal vesicle. Stroma and muscularis do not accept the stain. SCA is absent from the testicle, the epididymis, and the prostate. Stainable material is found in the lumen of the ejaculatory duct, but its epithelium remains unstained.

3040

Weil, A.J.; Rodenburg, J.M. 1962. The seminal vesicle as the source of the spermatozoa-coating antigen of seminal plasma. Proc. Soc. Exp. Biol. Med. 109:567-570.

Fluorescein-conjugated antiseminal plasma immune globulin stains specifically both human and rabbit seminal spermatozoa. Testicular spermatozoa lack reactivity with this reagent for SCA. The study of the components of the male genital tract of the rabbit indicates that SCA is produced in the seminal vesicle.

3050

Winnick, T.; Goldwasser, R. 1961. Immunological investigation of the origin of myosin of skeletal muscle. Exp. Cell Res. 25:428-436.

Rabbit antiserum against chicken myosin was labeled with fluorescein isothiocyanate and reacted strongly with chicken muscle mitochondria and microsomes. The staining could be inhibited by potassium chloride extract of myofibrils. Particulate fractions of chicken liver and pancreas did not bind the labeled antimyosin. In agar gel diffusion experiments, three distinct antigenic components were shown to be common for myosin, myofibrils, and muscle mitochondria and microsomes of chickens. One of these antigens was also detected in subcellular fractions of pancreas and liver and in blood plasma. The results support the view that myosin, and probably other myofibrillar fractions, are synthesized in the granules of the muscle cell. It is postulated that they are then released and integrated to constitute the myofibril structure.

306C

Yeager, J.A.; Anderson, T.O. 1959. Localization of testicular hyaluronidase using fluorescent antibody. Acta Anat. 39:189-197.

Using a double-layer fluorescent antibody technique, the antigens of a purified bull testicular hyaluronidase preparation were localized in sections of bull testis. Specific

fluorescence occurred in seminiferous epithelium in the region of spermatocytes and spermatogonia. In a tangential section of a reactive seminiferous tubule the distribution of fluorescence suggested its occurrence at the surfaces of spherical cells. Considering other pertinent data, the hypothesis was offered that spermatocytes synthesize hyaluronidase during periods of mitotic activity to disaggregate the cementing substance between cells, allowing displacement of differentiating cells toward the lumen of the tubule.

B. PATHOLOGY

3070

Anderson, P.J.; Cohen, S.; Barka, T. 1961. Hepatic injury: A histochemical study of intracytoplasmic globules occurring in liver injury. *Arch. Pathol.* 71:89-95.

Injury to the rat liver produces intracytoplasmic globules within hepatic cells adjacent to areas of necrosis. Globules are PAS-positive, stain positively for bound lipids, contain acid phosphatase, and are strongly fluorescent when treated with fluoresceinated rabbit antirat immune globulin. The PAS reaction is reduced or eliminated after acetylation, pepsin digestion, and lipid extraction. The globulins are reduced in size and number after starvation. Globules may result from enlargement of cytoplasmic structures, lysosomes, intracellular complex lipids and embedded plasma proteins.

3080

Andres, G.A.; Morgan, C.; Hsu, K.C.; Rifkind, R.A.; Seegal, B.C. 1962. Electron microscopic studies of experimental nephritis with ferritin-conjugated antibody: The basement membranes and cisternae of visceral epithelial cells in nephritic rat glomeruli. *J. Exp. Med.* 115:929-936.

Ferritin-conjugated antibody has been used to identify by electron microscopy the sites at which nephrotoxic globulins localize in rat kidney during acute experimental glomerulonephritis. Basement membranes were primarily involved. Data confirm results from earlier FA studies. Concurrent FA studies indicated that buffered 5 per cent formalin at pH 7.2 and 4°C was a superior fixative to acetone or to osmium tetroxide. Methacrylate treatment of tissue, even after removal, prevented FA staining.

3090

Berns, A.; Blumenthal, H.T. 1961. Fluorescence microscopy study of diabetic glomerulosclerosis. *Federation Proc.* 20:17.

Crystalline beef insulin and globulin fractions of the serum of human diabetics showing a strong binding capacity between iodine 131 and insulin were labeled with fluorescein isothiocyanate. Sections of kidneys showing the lesions of diabetic glomerulosclerosis were stained with the fluorescein-tagged insulin and intense fluorescence was noted in the nodular foci of the glomerular capillaries, the glomerular basement membrane, afferent and efferent arterioles, and the basement membrane of tubules. This fluorescence with fluorescein-tagged insulin could be partially inhibited by pretreating the sections with nonfluorescent insulin. Similar studies were carried out utilizing the sandwich technique and the fluorescein-labeled globulin preparations. Normal kidney and kidneys with other lesions were similarly studied as controls. The results indicate that the nodular lesions of the glomeruli in diabetes contain substances with a strong insulin-binding capacity.

110

3100

Broberger, O.; Perlmann, P. 1962. Demonstration of an epithelial antigen in colon by means of fluorescent antibodies from children with ulcerative colitis. *J. Exp. Med.* 115: 13-25.

Thirteen sera from children with ulcerative colitis were examined by FA for antibodies reacting with constituents of human colonic tissue. Three of 10 sera reacted positively by the indirect method with conjugates of rabbit antihuman gamma globulin. Specificity was confirmed by inhibition tests. Sixteen sera from healthy children and adults were negative. Staining capacity of sera correlated with hemagglutination titer when tested with sheep erythrocytes coated with extract of colon. Absorptions indicated that the stainable antigen also present in the extracts was useful for hemagglutination. In unfixed tissue sections, fluorescent antibodies were adsorbed onto the epithelial cells of the mucosa but not on epithelial basement membranes. Fluorescent H agglutinins were nonspecifically adsorbed onto the same mucosal structures of human colon as antibodies in sera of patients with ulcerative colitis. Fluorescent serum from patients with rheumatoid arthritis also stained sections of human colon but with different localization and on different antigens. Sera from patients with systemic lupus erythematosus or with the nephrotic syndrome, all hemagglutinin-positive, did not stain the sections of colon.

3110

Clayton, R.M. 1954. Localization of embryonic antigens by antisera labeled with fluorescent dyes. *Nature* 174:1059.

Antibodies labeled with fluorescent dyes have been used for histochemical localization of antigens in tissue sections. This method might be applied to locate tissue antigens during the course of development. Since the changes in relative distribution of antigens at different stages of development are of interest from the point of view of differentiation, a modification of the method, which enables one to study several antigens simultaneously, has been employed.

3120

Clayton, R.M.; Feldman, M. 1955. Detection of antigens in the embryo by labeled antisera. *Experientia* 11:29-31.

The detection of tissue antigens during embryogeny could suitably be done with fluorescent-labeled antibodies when the antigens were well characterized, but antigens against tissue extracts could be expected to show cross-reactions. These authors used antisera labeled with iodine 131. Anti-lens and anti-cardiac muscle antisera were used and frozen sections were made. Some cross-reactions with other tissues were noted. Intensity patterns varied with the age of the embryos and young mouse tissues tested. Autoradiographs of findings are presented.

3130

Cruickshank, B.; Stuart-Smith, D.A. 1959. Orchitis associated with sperm-agglutinating antibodies. Lancet 1:706.

The factor in patient serum responsible for agglutination of sperm is antibody. Such antibody seemed to react with autologous and homologous antigen and may be responsible for some orchitis cases. Further work is required to clarify the mechanisms involved. FA was used to test sera against sperm and testis sections. No localization of antibodies as noted.

3140

Dixon, F.J. 1960. Immunologic mechanisms in experimental vasculitis. Bull. N.Y. Acad. Med. 36:714.

Fluorescent antibody studies, as a part of the reported project, revealed the Arthus site to contain an antigen-antibody deposition in the subepithelium of the small vessels. In experimental glomerulonephritis of rabbits, a heavy antigen-antibody deposit was seen in the basement membranes of affected glomeruli.

3150

Fennell, R.H., Jr.; Reddy, C.R.R.M.; Vazquez, J.J. 1961. Progressive systemic sclerosis and malignant hypertension. Arch. Pathol. 72:209-215.

An immunohistochemical study of the renal lesions of progressive systemic sclerosis and malignant hypertension offers on one hand additional evidence for a morphologic similarity between the lesions of these two entities. The lesions in both diseases showed a preferential concentration of fibrinogen. On the other hand, a serologic difference may exist, in that three patients with malignant hypertension had no nuclear-localizing factors, but such factors may be found in patients with scleroderma. It is concluded that no definite interpretation in regard to pathogenesis of disease can be made in these two conditions.

3160

Fennell, R.H., Jr.; Santamaria, A. 1962. Immunochemical and histochemical study of hepatic lesions produced by anaphylaxis in the rat. Federation Proc. 21:305.

Adult Holtzman rats immunized by 3 injections of 5 mg of human serum albumin, HSA, in incomplete adjuvant developed titers of antibody detectable by hemagglutination and Ouchterlony plates. When shocked by injection into the tail vein of 5 mg of HSA most animals died in 4 to 12 hours but a few survived for 24 hours. Changes of severe congestion of periportal areas at 4 hours progressed to focal necrosis in 12 to 24 hours. Fluorescent antibody studies showed antigen preferentially localized to periportal sinusoids in early lesions and sharply localized to necrotic areas later. No lesions were seen in animals immunized but not shocked. Studies for oxidative enzymes showed reduction of activity in necrotic foci. Studies for acid phosphatase revealed a consistent sequence of changes in acid phosphatase granules of liver cells with early enlargement and formation of perinuclear clumps. Later granules disappeared and cytoplasm stained diffusely. Finally, loss of enzyme occurred in necrotic areas with simultaneous increase in enzyme activity in neighboring Kupffer cells.

3170

Hammer, D.; Barnett, J.; Dixon, F.J. 1962. Host factors in nephrotoxic serum nephritis. Federation Proc. 21:13.

These experiments were undertaken to determine the roles of heterologous antikidney serum and of host factors in the development of nephrotoxic serum nephritis, NSN. Normal rats and rats tolerant to rabbit gamma globulin, RGG, were injected with rabbit antirat kidney nephrotoxic serum, NTS. To induce tolerance, newborn rats were exposed to 20 mg of RGG intraperitoneally within 12 hours after birth and then daily for 1 week. One subsequent injection was given at 4 weeks of age. Rats 8 weeks of age received intravenously 1.0 ml NTS and a tracer dose of RGG labeled with iodine 131 simultaneously. Thirty-eight of 70 rats exposed to RGG at birth developed no proteinuria during the observation time of 12 weeks after injection. In control groups not exposed to RGG at birth the onset of severe proteinuria in 51 of 56 occurred within 12 hours after injection of NTS. In addition, rats of both groups responding with proteinuria began by the 3rd day to eliminate the labeled RGG rapidly; tolerant rats without proteinuria exhibited an extremely slow elimination. The rapid disappearance of labeled RGG from the blood of proteinuric rats was caused in part by loss in the urine but to a greater part by apparent immune elimination. In both tolerant nonproteinuric and control proteinuric rats immunofluorescent observations revealed prompt fixation of RGG in the glomerular basement membranes.

3180

Hulka, J.F.; Hsu, K.C.; Beiser, S.M. 1961. Antibodies to trophoblasts during the post-partum period. Nature 191:510-511.

FA was used to produce evidence favoring an immunologic interpretation for the finding of tagged globulin from post-partum sera in the syncytiotrophoblastic cytoplasm.

3190

Hunter, F.M.; Sparks, R.D.; Salzman, R.T. 1960. Autoagglutinins in hepatic disease. Gastroenterology 39:394-403.

When studied by a modification of the fluorescent antibody method, 3 of 4 patients with chronic progressive hepatic disease demonstrated circulating autoagglutinins. Six control patients and 2 patients with normal livers failed to demonstrate such agglutinins. These agglutinins possess some of the criteria for immunologic specificity usually demonstrated by antibodies. The autoagglutinins may have no pathologic significance; however, each patient who demonstrated autoagglutination had severe, progressive hepatic disease.

3200

Kaplan, M.H.; Craig, J.M. 1958. Production of cardiac lesions in rabbits immunized with heterologous heart tissue. Federation Proc. 17:2030:520.

Immunization of rabbits with homogenates of beef or rat heart stimulated antibodies reactive with normal rabbit heart tissue, as demonstrated by immunofluorescence and complement fixation. The antigen has been identified as an alcohol-soluble organ-specific constituent of the sarcoplasm of striated muscle. It may be differentiated by cross-absorption

tests from cardiolipin antigen. Rabbits given repeated injections of beef or rat heart homogenate incorporated in either alumina gel or Freund adjuvant develop small focal cardiac lesions characterized by myofiber necrosis and or interstitial inflammation. Rarely, an extensive myocardial fibrosis has been observed. The results of one experiment were as follows: Cardiac lesions were noted in 8 of 8 animals injected with rat heart, 7 of 9 animals given beef heart, and in 3 of 13 control rabbits injected with human gamma globulin. Myofiber necrosis was noted in 12 of the 17 experimental animals and in one of the 13 controls. In 7 of 15 experimental animals, bound gamma globulin could be detected within the sarcoplasms of myofibers, although all of these 15 rabbits showed elevated levels of circulating antibody. Myocardial cells are resistant to penetration by antibody to the sarcoplasmic antigen. Resistance may represent a limiting factor in pathogenesis of cardiac lesions.

3210

Kurtz, S.M.; Feldman, J.D. 1962. Morphologic studies of the normal and injured rat kidney following protein overload. *Lab. Invest.* 11:167-176.

Normal and nephrotic rats received intravenous or intraperitoneal injections of purified heterologous bovine serum albumin, BSA, so that the transit of this protein through the nephron could be studied. Normal animals developed numerous gram-stainable droplets scattered in the glomerulus and proximal convoluted tubules after 48 hours. Tissues prepared for fluorescent antibody studies and stained for the presence of BSA revealed that only the glomerular droplets were fluorescent. Electron micrographs showed that the glomerular droplets were accumulated in the visceral epithelial cell cytoplasm. The tubular epithelial cell droplets were accumulated in the visceral epithelial cell cytoplasm. They were readily visualized in the electron microscope and differed morphologically from those in the glomerulus. There was no evidence that tubular droplet formation was associated with the mitochondria of these cells. The nephrotic animals revealed essentially the same picture described above for the normal animals, except that the glomerular epithelial cells contained larger masses of droplets and clumps of the injected protein.

3220

Mancini, R.E.; Davidson, O.W.; Nemirovsky, M.S.; Vilar, O.; Bueno, M. 1961. Reaction of acrosomal germinal cells of guinea pigs and rats with fluorescent antitesticular antibody. *Rev. Soc. Argent. Biol.* 37:284-285. In Spanish.

More than half of the completely sensitized animals developed testicular lesions in the form of strophy of the germinal epithelium. Only 25 per cent showed circulating antibodies against testicle by complement fixation. The FA test demonstrated a specific fluorescence in the testicles against homologous globulin and acrosomal spermatids and the spermatozoid heads. Controls included blocking by normal globulins and absorption with lyophilized homologous testicle and yielded negative or feebly positive results. The same techniques applied to incompletely sensitized animals were negative. Lesions were not the same as in the first group. It is postulated that the reaction between immune serum globulin and the acrosoma indicates the existence in this structure of an antigenic substance found in testicular extract.

3230

Moran, T.J.; Kurtz, S.M.; Vazquez, J.J. 1962. Diabetic and cortisone-induced renal lesions, a morphologic and immunochemical study. *Lab. Invest.* 2:240-254.

Glomerular lesions in cortisone-treated rabbits have been studied by immunohistochemical techniques and by electron microscopy. A protein, probably a globulin and apparently derived from the circulating plasma, has been demonstrated as a prominent component of the lesions. By electron microscopy the lesions have been shown to be essentially intravascular, and no significant changes were found in basement membranes or epithelial, endothelial, or mesangial cells except in areas immediately overlying the nodular lesions. Globulin was also demonstrated in the glomeruli, the fibrin cap lesions, and the renal arterioles in cases of human diabetic glomerulosclerosis. Similarities and differences between the cortisone-induced lesions in rabbits and human diabetic glomerulosclerosis and the possibility of significance of demonstration of globulin in the human diabetic lesions have been discussed.

3240

Nairn, R.C.; Chadwick, C.S.; McEntegart, M.G. 1958. Fluorescent protein tracers in the study of experimental liver damage. *J. Pathol. Bacteriol.* 76:143-153.

Fluorescein-conjugated homologous whole serum or serum albumin was injected intravenously to provide a plasma tracer in rabbits and rats in which liver damage had been induced by the following methods: injection of renin after bilateral nephrectomy, to produce acute hypertension; acute carbon monoxide or anoxic asphyxia; carbon tetrachloride poisoning. The liver lesions varied from severe centrilobular necrosis, through cytoplasmic vacuolation, to minimal cytological damage not detected by routine histological methods. In every case, examination of unstained sections of these livers by ultraviolet fluorescence microscopy revealed an abnormal distribution of fluorescent tracer. Droplets of fluorescent material appeared in damaged liver cells. They were particularly conspicuous in the viable-looking cells that bordered areas of necrosis.

3250

Paronetto, F.; Schaffner, F.; Popper, H. 1961. Immunocytochemical reaction of serum of patients with hepatic diseases with hepatic structures. *Proc. Soc. Exp. Biol. Med.* 106:216-218.

Binding of sera of 32 patients with various liver diseases, ten patients with lupus erythematosus and 30 hospital controls to human and animal livers and other organs was studied by indirect fluorescent antibody. Two types of circulating reacting substances were demonstrated. One, noted in patients with primary biliary cirrhosis and malignant hepatitis, bound nuclei without species or organ specificity; the other, occurring in a variety of hepatic diseases, reacted with the cytoplasm of ductule cells. The former was influenced by steroid therapy, but the latter was not. The antigenic material in the ductular cell possibly is a muco- or lipoprotein that may also be present in bile.

3260

Scott, D.G. 1959. An immunohistological study of connective tissue. Ann. Rheum. Dis. 18:207-214.

The results are reported of an immunohistochemical analysis of connective tissue based on the use of two different antisera, each labeled with one of two contrasting fluorochromes, fluorescein or lissamine rhodamine RB 200. Basement membranes are antigenically distinct from reticulin on the one hand, and from a group of connective tissue components on the other. Members of this group include fibroblasts and fibrils in the interstitium of organs, in vascular adventitia, in proliferating fibrous tissue, and lying between bundles of collagen in the capsule of joints. Reticulin was antigenically related to, but not identical with, members of the third group of connective tissue components. Thus it appears that three connective tissue antigens, or groups of antigens, can be recognized by immunohistochemical means. All three groups react with antisera prepared against human glomeruli, but only two, reticulin and the third or fibrous tissue group, react with antisera prepared against human synovium. These findings do not necessarily imply that the renal glomerular basement membrane contains reticulin.

3270

Scott, D.G. 1960. Immunohistochemical studies of connective tissue: The use of contrasting fluorescent protein tracers in the comparison of two antisera. Immunology 3:226-236.

Two fluorochromes of contrasting color, fluorescein and lissamine rhodamine RB 200, have been used in comparing the reactions with their antigens in tissue sections of anti-human-glomerulus antisera and anti-human-synovius antisera. A uniform redistribution of labeled antibodies took place when tissue sections pretreated with fluorescein-labeled anti-glomerulus globulin were exposed to anti-glomerulus globulin labeled with lissamine rhodamine RB 200. In sections pretreated with an anti-human-glomerulus conjugate and exposed to an anti-human-synovium conjugate labeled contrastingly, and vice versa, the redistribution of antibodies did not occur to the same extent in every situation. There may be a difference in the stability of union between certain connective tissue antigens and antibodies present in anti-glomerulus conjugates, on the one hand, and anti-synovium conjugates on the other. The results of these experiments and of others in which anti-glomerulus and anti-synovium conjugates labeled contrastingly were allowed to react with tissue sections simultaneously, indicate that splenic reticulin is antigenically dissimilar to fibrils in vascular adventitia.

3280

Seegal, B.C. 1959. The value of the technique using fluorescent antibodies in the study of experimental nephritis: Its possibilities for application to an interpretation of the pathogenesis of certain forms of human nephropathy. Rass. Fisiopol. Clin. Terap. 31:12:1063-1078. In Italian.

This address is a review of work in experimental nephritis. The fluorescent antibody method is the principal study tool.

3290

Steblay, R.W. 1962. Glomerulonephritis induced in sheep by injections of heterologous glomerular basement membrane and Freund complete adjuvant. J. Exp. Med. 116:253-272.

Circulating serum antibodies that localized by FA on basement membrane structures of the heterologous donor kidney antigen or produced nephritis in the heterologous donor species were found in serum of sheep with nephritis. This represented the first passive transfer of nephritis by serum antibody to a heterologous species from an animal with nephritis. Aspects of pathology, timing, and clinical observations were discussed.

3300

Steblay, R.W. 1962. Localization in human kidney of antibodies formed in sheep against human placenta. J. Immunol. 88:434-442.

Sheep anti-HGBM, human glomerular basement membrane, and anti-human placenta antibodies, when applied *in vitro*, localize in identical manner in the basement membranes of glomeruli, tubules, capsules, intertubular capillaries, certain extracellular sites in the media, and adventitia of arteries; they have similar distribution patterns when applied to human placenta. Cellular structures are not stained. Both placenta and HGBM greatly decrease or eliminate fluorescence when used for adsorption. A similar or identical antigen, or a group of antigens, is present in the placenta, HGBM, and other connective tissue structures of the kidney. The sheep injected with HGBM and Freund adjuvant in this experiment developed an acute proliferative glomerulonephritis. Autofluorescent internal and external elastic laminae were never seen in placental blood vessels observed, even though some were of considerable size.

3310

Steiner, J.W. 1961. Investigations of allergic liver injury, light, fluorescent and electron microscopic study of the effects of soluble immune aggregates. Amer. J. Pathol. 38:409-426.

Soluble complexes of antigen and antibody composed of human serum albumin and rabbit antihuman serum albumin prepared *in vitro* were injected into the portal circulation of a group of normal rabbits. Theoretical conditions of antigen excess were created in another group of hyperimmune rabbits that received a large injection of specific antigen into the portal vein. The resulting midzonal necrosis of liver cells in both groups of animals was indistinguishable from the injury produced when precipitated particulate immune aggregates were administered. When fluorescent tracer-labeled antigen was used in these experiments, precipitates of this material were found in the necrotic areas even when soluble complexes were injected. The question of a possible ischemic basis for the necrosis remained unresolved.

3320

Taylor, H.E.; Shepherd, W.E.; Robertson, C.E. 1961. An immunohistochemical examination of granulation tissue with glomerular and lung antiserums. Amer. J. Pathol. 38:39-48.

Fluorescein antibody conjugate prepared against rat glomeruli and lungs reacted in vitro with a wide variety of naturally occurring reticulins but failed to react with the young argyrophilic fibers, reticulin, in areas of granulation tissue. This indicates a lack of antigenic relationship between these groups of argyrophilic fibers, which also differ in some respects chemically. The same conjugates reacted specifically with capillaries in many organs and with newly formed capillaries in the areas of granulation tissue. The reacting antigen seemed to be associated with the cytoplasm of the young capillary endothelial cells rather than with the basement membranes per se. When the same unconjugated antisera were injected intravenously, the only demonstrable site of localization was in the glomerular basement membranes.

3330

Wissler, R.W.; Kao, V. 1962. Immunohistochemical studies of the human aorta. Federation Proc. 21:95.

Multiple unfixed 4-micron sections from 18 aortic areas of 9 individuals, 2 to 70 years of age, were studied microscopically using fluoresceinated rabbit antibodies to immunoelectrophoretically pure human plasma fractions, including beta lipoprotein, alpha lipoprotein, albumin, and fibrinogen. The severity of the atherosclerotic lesions sampled varied from no evidence of disease, through fatty streak and fatty plaque to complicated lesions. Adjacent cryostat sections from each aortic area were stained with Sudan IV, various connective tissue stains, and fibrin stains. Suitable control sections were studied. Immunoelectrophoresis, as well as positive staining of model streaks, demonstrated the avidity of the labeled antibodies. The fluorescent anti-beta lipoprotein consistently stained diseased areas of aortic intima where lipid was revealed by fat stain. Fatty streaks and plaques tended to stain more intensely than the necrotic centers of complicated lesions. The fluorescent anti-alpha lipoprotein either stained the diseased areas very faintly or not at all. The fluorescent-antihuman fibrinogen stained small focal areas of intima and adjacent media. The areas stained for fibrin immunohistochemically were more numerous in the complicated lesions and were absent in several of the fatty streaks studied. The fluorescent-antihuman albumin stained none of the lesions.

VII. OTHER TISSUE SUBSTANCES

3340

Arquilla, E.R.; Coblenze, C. 1960. The isolation of rabbit insulin antibodies. Anat. Rec. 138:203-209.

It has been possible to prepare an insoluble insulin aggregate by conjugating insulin to a cellulose stroma complex with bisdiazobenzidine. This complex can absorb insulin antiserum. Insulin antibodies may be isolated in a 500-fold purity from this aggregate. There are a multiplicity of antibodies to the insulin molecule that have a multiplicity of binding characters.

3350

Baum, J.; Simons, B.E.; Unger, R.H.; Madison, L.L. 1962. Localization of glucagon in the alpha cells in the pancreatic islet by immunofluorescent technics. Diabetes 11: 371-374.

Localization of the site of glucagon production has been indirect in all previous studies. In this work immunofluorescent techniques were used to identify the site of origin of glucagon. A specific rabbit antipearl-pork glucagon antiserum was used to identify glucagon in the pancreatic islet. Fluorescein isothiocyanate-labeled goat anti-rabbit antiserum was the fluorescent tag that permitted visualization of the site of the antiglucagon-glucagon reaction in bovine pancreas. Appropriate control studies demonstrated the specificity of the reaction. In serial sections alpha cells were located by the darkfield illumination technique. The fluorescent tag showed glucagon to be located in those cells identified as alpha cells in adjacent 4-micron sections. This could be differentiated from the beta cells identified by anti-insulin antiserum. These data provide the first direct evidence for the production of glucagon in the alpha cells of the pancreatic islet.

3360

Costarzi, G.; Mancini, A.M.; Zampa, G.A. 1961. Experimental research on insulin antibody: III. Immunohistology of pancreas with guinea pig anti-bovine insulin serum conjugated with fluorescein isothiocyanate. Boll. Soc. Ital. Biol. Speriment. 37:24:1233-1235. In Italian.

Serum reacted with various indigenous insulins present in tested pancreases. Insulins demonstrated different antigenic behavior, not entirely species-specific. The most intense specific staining obtained with a conjugate directed against bovine anti-insulin was obtained in bovine pancreas. Staining was of less intensity in the pancreas of sheep, an animal specifically related to cattle; it was still less in the pig and the horse, and was absent in the other mammals examined. The hyperglycemia-producing effect, observed in the rabbit with guinea pig anti-insulin serum, purified and conjugated, contrasted to the absence of staining in the endocrine pancreas of the rabbit by the same serum. This discrepancy has been confirmed. Three hypotheses were formed: Presence of different points of attack of a single anti-insulin antibody on insulin, with formation of bonds neutralizing the hormones that are different from those in other

animal species; formation of different antibodies, some less specific, others more specific; and necessity for bond energy between antibody and antigen greater for identification than for neutralization.

3370

Costanzi, G.; Mancini, A.M.; Zampa, G.A. 1962. Experiments on insulin antibody: IV. Morphology by immunohistology of lymphoid tissue and spleens of guinea pigs immunized with insulin. *Boll. Soc. Ital. Biol. Sperim.* 38:13:631-637. In Italian.

Pathology due to antigen-antibody reaction was demonstrated. Insulin resistance was demonstrated immunologically.

3380

Craig, J.M. 1962. The localization of ferritin in the tissues of newborns by immuno-fluorescent techniques. *Amer. J. Dis. Child.* 104:552-553.

Ferritin was isolated by the cadmium sulfate technique of Granick from human liver and spleen. It was reprecipitated 3 times in 10 per cent cadmium sulfate. After dialysis it was suspended in incomplete Freund adjuvant and administered to rabbits for antibody production. The resulting antisera gave a single line in the Ouchterlony plate against the antigen and gave no line when tested against normal human sera. Labeled fluorescent antisera preparation and tissue staining were carried out by the Coons technique with appropriate controls. Localization of ferritin was demonstrated in the reticuloendothelial cells of lymph nodes, the spleen, and the Kupffer cells of the liver. It was found in the parenchymal cells of the liver on occasion and in the brush border and the cytoplasm of the proximal tubule of the kidney. In none of these areas was ferritin uniformly demonstrable. When present it was often accompanied by histologically visible iron deposits. It often appeared prominently in infants with erythroblastosis. Repeated attempts to demonstrate ferritin by this method in the immature and term placenta have failed.

3390

Cruickshank, B.; Currie, A.R. 1956. Localization of tissue antigens with the fluorescent antibody technique: Application to human anterior pituitary hormones. *Immunology* 1:13-26.

Antisera have been prepared to adrenocorticotrophic hormone, thyrotrophic hormone, and growth hormone extracted from human pituitaries and to gonadotrophic and thyrotrophic hormone extracted from human urine. Precipitin, absorption-precipitin, and gel-diffusion tests have been performed with these antisera. No conclusive evidence was obtained of the presence of a hormone-specific antibody in any of the antisera. Cross-reactions occurred indicating presence of an antigen common to extracts prepared from the pituitary and a further antigen, or antigens, common to extracts prepared from the urine. Despite absence of hormone-specific antibodies, fluorescein-labeled samples of the antisera gave staining in cytoplasm of cells in the anterior pituitary and did not stain other tissues. Interpretation of tissue localization studies with labeled antisera is discussed.

3400

De Baroieri, A.; Scevola, M.E. 1958. Preparation of some fluorescent enzymes and measurement of their biochemical activity. *Boll. Ital. Biol. Sperim.* 34:19:1189-1192. In Italian.

Determination of the specific activity of the protein enzymes cytochrome c, ribonuclease, trypsin, and chymotrypsin coupled with fluorescein isocyanate has demonstrated that cytochrome c loses 23.5 per cent of its initial activity, ribonuclease loses 63 per cent, and trypsin and chymotrypsin are completely inactivated.

3410

Goodfriend, L.; Leznoff, A.; Sehon, A.H. 1961. Antibodies to estrone protein conjugates: III. Tissue localization of estrogens. *Can. J. Biochem. Physiol.* 39:957-971.

Evidence was obtained that the mature rat ovary contained cellular components that cross-reacted with fluorescein-labeled antiserum to estrone-2-carbamido-HSA.

3420

Halikis, D.N.; Arquilla, E.R. 1961. Studies on the physical, immunological, and biological properties of insulin conjugated with fluorescein isothiocyanate. *Diabetes* 10: 142-147.

A fluorescein isothiocyanate derivative of insulin was prepared, and its biological, immunological, and physical properties were investigated. Fluorescent insulin has at least two components by electrophoresis, each of which migrates faster than crystalline insulin. The fluorescence of insulin labeled with fluorescein is augmented by serum proteins. Insulin conjugated with fluorescein isothiocyanate reacts immunologically to a lesser degree than crystalline insulin when exposed to rabbit antibody against crystalline insulin as measured by hemagglutination. This altered activity could not be demonstrated in the cross-inhibition of hemagglutination. The biological activity of insulin in Wistar rats was significantly decreased when conjugated with fluorescein isothiocyanate.

3430

Hartley, B.S.; Massey, V. 1956. The active center of chymotrypsin: I. Labelling with a fluorescent dye. *Biochem. Biophys. Acta* 21:58-70.

1-Dimethylaminonaphthalene-5-sulphonyl chloride reacts with α -chymotrypsin, chymotrypsinogen, and DIP-chymotrypsin to give fluorescent conjugates. The absorption spectra and fluorescent properties of these conjugates have been investigated. The enzyme activity of α -chymotrypsin is inhibited by reaction with one molecule of the fluorescent dye. Competitive inhibitors will protect the activity of the enzyme during reaction with the dye. The dye-labeled enzyme will no longer react with organo-phosphorus inhibitors. It can be reactivated by hydrolysis at alkaline pH. It is concluded that the dye reacts with a group in the active center of the enzymes. Dye-labeled chymotrypsinogen can be activated with trypsin to give fully active dye-labeled chymotrypsin. The stability and fluorescent properties of dye-amino-acid derivatives have been

compared with dye-labeled chymotrypsin. Dye-labeled imidazole rings bear the closest resemblance to the labeled enzyme. The significance of these findings with respect to the chemical nature of the active center of chymotrypsin is discussed.

3440

Hartroft, P.M.; Edelman, R. 1960. Renal juxtaglomerular cells in sodium deficiency; effect of hypophysectomy; localization of renin by the fluorescent antibody technique, p. 63-68. In J.H. Moyer and M. Fuchs, ed. A Hahnemann symposium on salt and water retention: Edema mechanisms and management. W.B. Saunders Company, Philadelphia.

Evidence from fluorescent anti-renin, together with previous results, strongly supports elaboration of renin by juxtaglomerular cells. Final proof awaits technique improvements and renin purification.

3450

Kent, S.P. 1961. A study of mucins in tissue sections using the fluorescent antibody technique: I. The preparation and specificity of bovine submaxillary gland mucin antibody. *J. Histochem. Cytochem.* 9:491-497. Also *Federation Proc.* 20:16, 1961.

Mucin was extracted from bovine submaxillary glands and purified by a modification of the Sevag procedure. Antibodies to the mucin were produced in rabbits. The antibodies were labeled with fluorescein and used to demonstrate bovine submaxillary gland mucin in routine paraffin sections of formalin-fixed tissues. The fluorescent-labeled antibody did not react with the submaxillary gland of the pig, dog, mouse, rat, rabbit, or human or with other mucin-containing organs of cattle. This indicated that the antibodies are organ-specific as well as species-specific. The results of the study further suggest that epithelial mucins from other tissues may also stimulate antibodies with high specificity.

3460

Kent, S.P. 1962. A study of mucin in tissue sections using the fluorescent antibody technique: II. The preparation and specificity of human submaxillary gland mucin antibody. *Federation Proc.* 21:15.

The preparation of organ- and species-specific antibodies to bovine submaxillary gland mucin labeled with fluorescein and used to study mucin in paraffin-embedded tissue sections was recently reported. Similar studies with human submaxillary glands have been performed. The mucin was extracted and purified by a modification of the Sevag procedure. Antibodies to the mucin were produced in rabbits, labeled with fluorescein isothiocyanate, and used to demonstrate human submaxillary gland mucin in paraffin sections of formalin-fixed tissues. The fluorescein-labeled antibody reacted with the submaxillary gland mucin in the twelve human specimens studied but did not react with the submaxillary gland mucin of the monkey, cow, pig, dog, rat, mouse, rabbit, or guinea pig. Cross-reactions were observed with the mucin of human tracheal, sublingual, lingual, cervical, and Bartholin glands. The surface mucin of the stomach, ileum, and gall bladder also reacted. Mucin of colon and mammary ducts did not show specific fluorescence when exposed to the labeled antibody.

3470

Lacy, P.E. 1959. Electron microscopic and fluorescent antibody studies on islets of Langerhans. *Exp. Cell Res. Suppl.* 7:296-308.

The ultrastructure of the beta granules is distinctly different in the islets of the rat, dog, cat, rabbit, guinea pig, and chicken. The variations are so characteristic that each species can be identified on the basis of the ultrastructure of its beta granules. Endogenous insulin was demonstrated in the beta cells by fluorescent-labeled antibodies to beef insulin. The beta cells of the rat, dog, cat, rabbit, and chicken were stained with the labeled antibodies to insulin, indicating that the endogenous insulin of these species was not immunologically different from beef insulin. The beta cells of the guinea pig did not react with the fluorescent-labeled antibodies to insulin. A uniform ultrastructure of secretory granules of alpha cells was observed in the different species.

3480

Lacy, P.E.; Davies, J. 1957. Preliminary studies on the demonstration of insulin in the islets by the fluorescent antibody technic. *Diabetes* 6:354-357.

The globulin fraction of guinea pig serum, containing antibodies to insulin, was labeled with fluorescein isocyanate. The labeled, anti-insulin globulin was used to stain frozen sections of pancreas from the beef, cat, mouse, rat, guinea pig, rabbit, and man. A yellow-green fluorescence was produced in the islets of the beef, cat, rat, and mouse pancreas by this labeled globulin fraction. The fluorescent reaction was inhibited in the beef and mouse pancreas by the prior addition of either commercial insulin or crystalline pork insulin to the labeled globulin. The fluorescent reaction was not inhibited by the addition of glucagon. Fluorescence was not produced in the islets of the guinea pig, rabbit, and human pancreas.

3490

Lacy, P.E.; Davies, J. 1959. Demonstration of insulin in mammalian pancreas by the fluorescent antibody method. *Stain Technol.* 34:85-89.

An improved technique is presented for demonstrating insulin location in tissue sections. Detailed instructions are given for antiserum preparation, tissue freeze-drying, embedding, sectioning, staining, examination, and photography.

3500

Levi-Montalcini, R.; Angeletti, P.U. 1961. Growth control of the sympathetic system by a specific protein factor. *Quart. Rev. Bio.* 36:99-108.

The growth, differentiation, and maintenance of the sympathetic nerve cells were found to depend upon a specific protein factor. The chemical, biological, and immunological properties of this factor are discussed in connection with the recent findings of the presence of the growth factor in the sympathetic nerve cells of a variety of mammals, including man.

3510

Leznoff, A.; Fishman, J.; Goodfriend, L.; McGarry, E.E.; Beck, J.C.; Rose, B. 1960. Localization of fluorescent antibodies to human growth hormone in human anterior pituitary glands. Proc. Soc. Exp. Biol. Med. 104:232-235.

Use of fluorescent antibody has shown that rabbit antibodies to Raben human growth hormone localize specifically in cells of the human anterior pituitary gland. This antibody probably localizes exclusively in eosinophil cells of normal human pituitaries and in eosinophilic adenomata from patients with acromegaly.

3520

Leznoff, A.; Fishman, J.; Goodfriend, L.; McGarry, E.E.; Rose, B.; Beck, J.C. 1960. The cytological localization of human growth hormone with fluorescent antibody. J. Clin. Invest. 39:1006-1007.

Using gel diffusion and hemagglutination techniques, our laboratory and others have shown that antisera to the Raben more highly purified preparation of human growth hormone are species-specific and hormone-specific. Antisera to human growth hormone were conjugated to fluorescein isothiocyanate and applied to sections of normal human pituitaries, eosinophilic adenomata, and an unclassified pituitary adenoma from a patient with severe Cushing disease. The fluorescent antisera localized almost exclusively in pituitary eosinophilic cells and in eosinophilic adenomata. There was no staining of other organs or adenomata. The staining was inhibited with antihuman growth hormone and absorbed by prior incubation with human growth hormone. Unstained sections and sections stained with heterologous conjugates showed no fluorescence. Fluorescent antihuman gamma globulin localized in blood vessels and fibrous septa of the pituitary. These studies contribute evidence for the specificity of our antisera to human growth hormone and evidence that the pituitary eosinophil is the cellular site of production or storage of growth hormone in the human.

3530

Mancini, A.M.; Zampa, G.A.; Costanzi, G. 1961. Experimental research on insulin antibody: II. Immunochemical characterization of guinea pig anti-insulin serum conjugated with fluorescein isothiocyanate. Boll. Soc. Ital. Biol. Sperim. 37:24:1230-1232. In Italian.

Anti-insulin antibody activity of guinea pig serum is carried out by the gamma globulins. The hyperglycemia and glycosuria obtained in the rabbit with anti-insulin guinea pig serum against bovine insulin can be attributed to gamma globulin. Conjugation of gamma globulin contained in the guinea pig serum with fluorescein isothiocyanate does not modify the anti-insulin antibody. Blocking of insulin by specific antibodies, at least in the guinea pig, can be demonstrated immunologically only by the hemagglutination test.

3540

Mancini, E.E.; Vilar, O.; Dellacha, J.M.; Gimeno, A.; Castro, A. 1959. Histological localization of fluorescent thyroid-stimulating hormone in rat tissues. *Nature* 184: 1733-1734.

After labeling with lissamine rhodamine RB 200, thyroid-stimulating hormone retained part of its biological activity. Tracing studies thus provided new data on histological dynamics and localization of the hormone.

3550

Mancini, R.E.; Davidson, C.W.; Dellacha, J.C.; Alvarez, B. 1961. Participation of the kidney in the incorporation of fluorescent proteins and pituitary hormones. *J. Histochem. Cytochem.* 9:614-615.

Various hormones and serum proteins were labeled with fluorescent dyes and injected into rats and the distribution was traced. Free fluorescent dyes injected as controls were generally not retained. In the first few minutes, albumin, globulin, and fibrinogen occurred in small amounts in capillaries of the glomeruli, then in the lumina of the Bowman capsules, the proximal convoluted tubules, the intertubular capillaries of the cortex, and in the profuse capillary bed of medulla. After 6 hours they began to disappear from the glomerulus and capillaries, and at 8 hours they appeared in the apical portion of the cells of proximal convoluted tubules. There they slowly increased up to 48 hours, remained 6 to 8 days, and then disappeared. Pituitary hormones appeared faster and in greater amount in the same sites of the nephron, reaching their maximal accumulation in 6 hours and disappearing after 48 hours. Small amounts of these hormones were present in the lumina of the distal convoluted and collecting tubules, and also showed reabsorption by some of the epithelial cells.

3560

Marshall, J.M. 1951. Localization of adrenocorticotrophic hormone by histochemical and immunochemical methods. *J. Exp. Med.* 94:21-29.

The fluorescent antibody technique was adapted to the localization of native protein antigens in cells and tissues. This method was applied specifically to the localization of adrenocorticotrophic hormone in the pituitary gland. An antiserum to hog ACTH was produced in an adrenalectomized rabbit. The alpha-2-globulin fraction of the serum was conjugated with fluorescein. After purification, the fluorescent antibody solution stained selectively the cytoplasm of basophil cells of the hog pituitary. No cells of sheep or beef pituitary or of hog kidney were stained. A fluorescent globulin solution prepared for normal rabbit serum gave no selective staining in any of these tissues. Immunochemical tests showed that the fluorescent antibody gave a precipitin reaction with a highly active ACTH preparation of low molecular weight. The supernatant solution from this reaction showed a loss of hormone activity.

3570

Marshall, J.M. 1954. Distributions of chymotrypsinogen procarboxypeptidase, deoxyribonuclease, and ribonuclease in bovine pancreas. *Exp. Cell Res.* 6:240-242.

One- to 2-micron sections of beef pancreas were prepared. Each was treated with one of the labeled antibody solutions. As controls, other sections were treated with labeled globulin from normal rabbits. When the sections were examined in the fluorescence microscope, the staining pattern of each of the antibody solutions was sharply specific. Adjacent sections, in which individual acinar cells could be studied serially, revealed most accurately the differences in distribution of the four antigen preparations.

3580

Menechelli, V.; Scapinelli, R. 1962. The site and time of onset of growth hormone production in the bovine adenohypophysis, determined by the fluorescent antibody method. *Acta Anat. Basel* 51:198-208.

In bovine adenohypophyses of embryos, foetuses, and adults of various ages the production of growth hormone was investigated by the fluorescent antibody method. As in humans, the cells responsible are the acidophils. Growth hormone production was earliest demonstrable in foetuses of 18 cm, that is, at about the 3rd month of pregnancy, and increased rapidly thereafter. By the adult stage, the nonfluorescent acidophils had become predominant; these are probably resting cells and the change represents the end of somatic growth. The possibility that some acidophils are nonfluorescent because they produce other hormones is mentioned.

3590

Midgley, A.R.; Pierce, G.B. 1962. Immunohistochemical localization of human chorionic gonadotropin. *J. Exp. Med.* 115:289-294.

By immunohistochemical techniques, human chorionic gonadotropin has been localized to syncytiotrophoblastic cells of immature placenta, hydatidiform mole, choriocarcinoma, and choriocarcinoma. No gonadotropin has been detected in cytotrophoblast. Evidence suggests that syncytiotrophoblast is the cell of origin of human chorionic gonadotropin. The observation that formalin fixation did not alter the ability of human chorionic gonadotropin to react with its specific antibody permitted the study of formalin-fixed paraffin-embedded tissues stored in the tissue collection. In addition, the excellence of histologic preparations following formalin fixation facilitated cytologic identification.

3600

Moon, H.D.; McIvor, E.C. 1960. Elastase in the exocrine pancreas: Localization with fluorescent antibody. *J. Immunol.* 85:78-80.

The enzyme elastase was demonstrated in the acinar tissue of the porcine pancreas, using antielastase rabbit serum and fluorescent sheep antirabbit globulin to identify and localize the enzyme. No elastase was present in the islets of Langerhans. The specificity of the antibody to porcine elastase was demonstrated by its strong reaction with porcine

pancreas and its failure to react with human or guinea pig pancreas. A positive reaction occurred between guinea pig anti-insulin serum and the islets of Langerhans of porcine pancreas, but there was no reaction between this serum and the acinar tissue and ductal epithelium of porcine pancreas.

3610

Nace, G.W.; Suyama, T. 1961. The echotechnique: A procedure for the cellular localization of specific enzymes and other antigen utilizing preliminary agar diffusion with fluorescent antibody. *J. Histochem. Cytochem.* 9:596.

This technique separates and identifies multiple antigens from tissues without need for prior purification. The method is generally described in which fluorescent antibody is used in an agar diffusion or immuno-electrophoretic pattern to identify the desired antigen component of the system. The resultant complex is removed from the system, dissociated, and the FA used to stain tissue sections.

3620

Nairn, R.C.; Fraser, K.B.; Chadwick, C.S. 1959. The histological localization of renin with fluorescent antibody. *Brit. J. Exp. Pathol.* 40:155-163.

An antirenin serum was prepared by immunizing rabbits with a purified extract of pig renin in Freund adjuvant. Frozen sections of pig kidney were treated with antiserum. The antiserum was traced with a goat antirabbit globulin labeled with lissamine rhodamine RB 200, and the sections were examined by fluorescence microscopy. The good contrast of the tracer with the tissue autofluorescence and sensitivity of the sandwich method permitted the detection of specific fluorescence in the kidney after repeated immunological absorptions of the antirenin serum. Staining with unabsorbed antiserum was mainly glomerular, located in the epithelial cells. Absorptions with pig tissue, including kidney cortex preparations, or with renin extract at optimum precipitating proportions, left a proportion of antirenin activity. This antibody is nonprecipitating. Glomerular staining was blocked by neutralizing the antirenin activity with renin extract. Absence of staining with fully absorbed antiserum in sections of pig liver, lung, adrenal, and heart, and absence of kidney staining with normal rabbit serum were tests of specificity. Antibody in antirenin serum was predominantly antiglomerular. Renin is located in the glomeruli, probably in the epithelial cells. This evidence seems strong, but it cannot be conclusive until pure renin is available for a specific blocking test.

3630

Salmon, J.; Lambotte, R.; Smolar, V. 1962. An immunofluorescent study of human amniotic fluid. *Arch. Int. Physiol. Biochem.* 70:731-734.

Immunofluorescence permitted localization of certain specific parts of human amniotic fluid in the epithelium of the amnion. The observations make a new contribution to the secretion of amniotic fluid as proposed by Keiffer.

3640

Steiner, R.F.; McAlister, A.J. 1956. Studies upon fluorescent insulin conjugates. Nav. Med. Res. Inst. 14:987-1010.

Measurements of excited lifetime and of degree of polarization have been made on fluorescent conjugates of insulin with 1-dimethylaminonaphthalene-5-sulfonic acid. These results indicate that several organic agents produce a limiting rotational kinetic unit of lower relaxation time than that found in water.

VIII. CELL FUNCTION

3650

Beck, J.S. 1962. The behavior of certain nuclear antigens in mitosis. *Exp. Cell Res.* 28:406-418.

Human sera containing autoantibodies against three distinct components of cell nuclei have been used with the fluorescent antibody technique to study the fate of their respective antigens during mitosis in HeLa cells. The distribution of deoxyribonucleoprotein is shown to follow the chromosomes, although a soluble nuclear protein and an unidentified constituent of the nucleolus do not take part in the formation of the chromosomes and are found as a halo surrounding the chromosomal plate. These antigens return to the nucleus during telophase.

3660

Sbarra, A.J.; Shirley, W.; Bardawil, W.A. 1962. Piggy-back phagocytosis. *Nature* 194:255-256.

Fluorescent-labeled rabbit gamma globulin was used to demonstrate a piggy-back phenomenon wherein fluorescent material was taken up by actively phagocytizing cells. The fluorescent material was taken up in conjunction with particles but not when particles were absent. Phagocytosis was inhibited by malonate. The application to getting materials into cells not found previously possible was discussed.

3670

Sbarra, A.J.; Shirley, W.; Bardawil, W.A.; Ouchi, E.; Baumstark, J.J. 1962. Piggy-back phagocytosis. *Bacteriol. Proc.* M62:78.

The term piggy-back describes the phenomenon by which impermeable compounds gain entrance to cells after phagocytosis. This can be demonstrated with certain inhibitors or fluorescent-labeled compounds. Respiratory activity of resting cells is insensitive to malonate; however, the addition of equivalent concentrations of malonate to these cells during phagocytosis completely inhibits the increased respiratory activity associated with phagocytosis. Adding the malonate before the particles, washing the cells to remove exogenous inhibitor, and then adding the particles, results in increased oxidation normally associated with phagocytosis. These results further show that malonate does not penetrate in the absence of particles. The possibility of malonate entering the cell in a piggy-back fashion led to the following experiment: Fluorescent-labeled rabbit gamma globulin was offered to white cells in the absence and presence of inert particles. More fluorescence was observed in the cells that had engulfed particles. Particles did not become coated with the material. Fluorescent-labeled gamma globulin entered the cell through the same channel or vesicle that had formed at the cell surface of the phagocytizing cell.

130

3680

Went, H.A.; Mazia, D. 1959. Immunochemical study of the origin of the mitotic apparatus. *Exp. Cell Res. Suppl.* 7:200-218.

Eggs and embryos of the sea urchin were used to study the isolated mitotic apparatus. Chemical analysis of proteins failed to identify the proteins of the mitotic apparatus. Immunochemical approaches used were the Ouchterlony technique and fluorescent antibody. The Ouchterlony technique revealed only one unfertilized egg antigen shared with the mitotic apparatus, and conversely the mitotic apparatus contained no antigens not present in the unfertilized egg. There was no antigenic difference between the normal mitotic apparatus and that produced by parthenogenesis. Thus the hypothesis that the mitotic apparatus is formed from materials already present in the cell at the time of division seems to be upheld. This apparatus apparently contains a single major antigen. Fluorescent antibody studies confirmed that the antigen of the mitotic apparatus combined with an antiserum to the cell precursor component. This antigen appears to be localized in the cytoplasm of the blastula stage. FA will probably be useful in studying further development of the embryo.

3690

Zlotnick, A.; Vazquez, J.J.; Dixon, F.J. 1962. Mitotic activity of immunologically competent lymphoid cells transferred into X-irradiated recipients. *Lab. Invest.* 11: 493-499.

On the basis of mitotic rates in this study, antibody-containing cells in cell transfer sites could be derived from precursors through successive divisions. In the cell transfer system, antibody production is preceded by mitotic activity of the transferred lymphoid cells in a manner similar to that of the lymphoid tissue of the intact animal.

IX. NEOPLASMS

A. DIFFERENTIATION

3700

Calabresi, P.; Edwards, E.A.; Schilling, R.F. 1959. Fluorescent antiglobulin studies in leukopenic and related disorders. *J. Clin. Invest.* 38:2091-2100.

A method for studying leukocyte immunology by the fluorescent antiglobulin technique is described. The results with sera from various disease states are reported. The data presented are consistent with the concept that human antileukocyte globulins may be directed against the nucleus or against the cytoplasm. Antinuclear globulins were detected in the sera of all patients with systemic lupus erythematosus, SLE, and the Felty syndrome studied, and in two cases of apparently uncomplicated rheumatoid arthritis. These findings suggest that the Felty syndrome may be a connecting link of a disease spectrum involving simple rheumatoid arthritis and SLE. Evidence that a circulating factor present in patients with Felty syndrome may be involved in the pathogenesis of the leukopenia was obtained in two instances by plasma transfusions.

3710

Calabresi, P.; Finch, S.C. 1962. The value of the fluorescent antiglobulin technic in the differential diagnosis of aleukemic leukemia. *Proc. 8th Congr. Int. Soc. Blood Transfus.* Tokyo. 364 p.

FA was applied to leukopenic patients to differentiate aleukemic leukemias from other conditions. Antinuclear factors were demonstrated in patients with a variety of disorders. The presence of antinuclear globulin in leukopenic patients may exclude diagnoses of aleukemic leukemia and lymphosarcoma.

3720

Chen, H.Y. 1962. Studies on the staining reaction of the fluorescent conjugate of globulin. *Shih Yen Sheng Wu Hseuh Pao* 7:283-298. In Chinese.

A review of past work is presented. The staining of sections of various tissues and tissue cultures were studied with respect to staining intensity under various conditions of pH, ionic strength of diluents, fixation methods, and effects of normal versus tumorous tissues. An interesting point was the use of pH 12.5, which gave very intense staining and good differentiation between the nucleus and the cytoplasm of cells. The dyes DANS and RB 200 were used for conjugation.

3730

Clayton, R.M. 1960. Labeled antibodies in the study of differentiation, p. 67-68. In P.B.M. Walker, ed. *New approaches in cell biology*. Academic Press, London and New York.

Fluorescent-labeled antisera can be of great advantage in studying tissue differentiation. Problems include determination of sites of compound synthesis during ontogeny, changes in carcinogenesis, reduction, regeneration, prolonged tissue culture, and many other problems. This review discusses the problems and some possible solutions using labeled antisera.

3740

Cohen, S.; Beiser, S.M.; Hsu, K.C. 1961. Immunohistochemical study of the uptake of serum proteins by neoplastic liver cells. *Cancer Res.* 21:1510-1512.

The uptake of various exogenous and endogenous serum proteins by neoplastic liver cells was demonstrated with fluorescein-tagged antibodies to these proteins. The imbibed proteins were found in clusters of intracytoplasmic globules with marked variation in size. In hematoxylin- and eosin-stained sections these globules appeared as round, refractile, and highly eosinophilic inclusions. This distribution contrasted sharply with that observed in Kupffer cells of non-neoplastic liver, where imbibed protein was found only in the form of small granules uniformly dispersed throughout the cytoplasm. Normal liver parenchymal cells did not take up protein.

3750

Coons, A.H. 1957. The application of fluorescent antibodies to the study of naturally occurring antibodies. *Ann. N. Y. Acad. Sci.* 69:658-662.

This is the first instance of a clear cytological demonstration of an antigenic difference between normal and malignant cells. It does not establish the existence of a different antigen in tumor cells, but it implies a difference in their antigenicity because, as they have lost a normal antigen, they have probably gained an abnormal one. The author indicates both the possibilities and the difficulties inherent in attempting to use immunohistochemical methods as an approach to the investigation of malignant tissue. Further details and bibliography are given in a review.

3760

Cormack, D.E.; Easty, G.C.; Ambrose, E.J. 1961. Interaction of enzymes with normal and tumor cells. *Nature* 190:1207-1208.

Wheat germ lipase was labeled with fluorescein and rhodamine RB 200. The resulting conjugates had half of the esterase activity of the original lipase. The conjugates were used to study pinocytotic activity of normal hamster kidney cells and hamster kidney tumor cells *in vitro* and mouse ascites cells *in vivo*. Altered properties of the tumor cells were noted.

3770

Engelhardt, N.V. 1962. Study of monospecific antitumor sera by the method of fluorescent antibodies. *Univ. Int. Contra Cancrum Acta* 18:94-96.

After elimination of cross-reacting antibodies, antibodies against soluble and insoluble antigens of an induced hepatoma of C3 HA mice are demonstrable. Antibody against soluble antigen was eluted from the specific precipitate. This antibody was monospecific by FA test.

3780

Ghose, T.; Nairn, R.C.; Fothergill, J.E. 1962. Uptake of proteins by malignant cells. Nature 196:1108-1109.

Direct and indirect FA methods were used to study uptake by various tumors. The only normal cells taking up the conjugates were Kupffer cells and phagocytes of the spleen. Ascites tumor cells and solid neoplasms both readily acquired the stains. The process was distinct from the property of cells to sequestrate amino acids.

3790

Hiramoto, R.; Bernecky, J.; Jurandowski, J.; Pressman, D. 1960. Fibrin in human tumors. Cancer Res. 20:592-593.

Sections of human tumors have been stained for fibrin deposits by the fluorescein label technique. Large clots of extravascular fibrin were demonstrated in most of the tumors tested. No such large clots were found in the several normal tissues investigated. However, there was some staining of certain areas of connective tissue in both normal and tumor tissues. The fibrin deposition in the spontaneous human tumors was very similar to that of the several transplanted rat tumors.

3800

Hiramoto, R.; Jurandowski, J.; Bernecky, J.; Pressman, D. 1961. Immunochemical differentiation of rhabdomyosarcomas. Cancer Res. 21:383-386.

When six rhabdomyosarcomas were stained with antisera by the fluorescein label technique three, classified as embryonal and usually difficult to diagnose as rhabdomyosarcoma, appeared to contain myosin, since they reacted with antimyosin antibody. The remaining three, which were easier to classify as typical rhabdomyosarcomas histologically, appeared to contain no myosin. Antiserum to connective tissue gave different results in that the embryonal cells did not contain connective tissue antigenic components, but the typical rhabdomyosarcoma cells did except for one of the adult rhabdomyosarcomas that only partially stained. The question arises whether the adult muscle tumors are of muscle origin or of stromal origin. If these tumors come from skeletal muscle cells, they must have lost the ability to synthesize myosin in amounts detectable by fluorescent antibody.

3810

Hiramoto, R.; Pressman, D. 1957. Immunohistochemical staining properties of human skin and some related tumors. Cancer Res. 17:1135-1137.

The immunohistochemical staining properties of sera against human epidermis and a malignant melanoma were studied. Both sera stained melanoma cells, neurilemma, connective tissues, and the papillary region of skin. The sera differed, however, in that only the anti-epidermis stained epidermis, hair follicles, mucoepidermoid tumor cells, and parotid tumor cells.

134

3820

Hughes, P.E.; Louis, C.J. 1959. Differential staining of normal and neoplastic tissue with fluorescein egg albumen. A.M.A. Arch. Pathol. 68:508-512.

A method for preparing conjugates of fluorescein isocyanate and egg albumen and the results of staining frozen sections prepared from the margins of tumors are described. In common with many other conjugates prepared from different serum protein fractions from various animals, fluorescent egg albumen has been found to stain non-neoplastic, but not neoplastic, tissues.

3830

King, E.S.J.; Hughes, P.E.; Louis, C.J. 1958. The species nonspecificity of globulins in the globulin-fluorescein staining of tissues. Brit. J. Cancer 12:5-13.

Following injection of rabbits with homogenates of rat tissue, a rabbit globulin combined with normal rat tissue cells but not with tumor cells of the same organ. This was demonstrated visually with a globulin fluorescein complex that fluoresced in ultraviolet light. Although at first regarded as an antigen-antibody reaction, it was shown, by the demonstration of the same characteristics when normal rabbit globulin was used instead of that from an injected rabbit, to be a protein interaction of no serological significance. This feature was examined first in the case of tumors produced in animals by the administration of chemical carcinogens but was applied subsequently to naturally occurring tumors of both animals and man. The stains were prepared by conjugating the globulins with both fluorescein and rhodamine without any significant difference being seen in the staining effect.

3840

King, E.S.J.; Hughes, P.E.; Louis, C.J. 1959. Differential fluorescence staining of normal and neoplastic tissue: Use of various serum proteins. Cancer 11:741-752.

Fluorescein and rhodamine gamma globulin conjugates stained normal but not neoplastic tissues. This was true regardless of species of globulin used, natural or induced tumor material, and previous immunization history of the globulin-donating animal. Albumin and alpha globulins react similarly. The phenomenon is true whether the tissue globulin system is homologous or heterologous. Therefore, this phenomenon cannot be explained serologically.

3850

Louis, C.J. 1957. Histochemical differences between regenerating and neoplastic rat liver. Australasian Ann. Med. 6:277-281.

Fluorescent sera obtained by conjugating fluorescein isocyanate with the globulin fraction of serum obtained from rabbits previously injected with homogenates of normal rat liver have an affinity for rat liver and regenerating rat liver but not for rat hepatome. There is a close correlation between these staining reactions and alterations occurring in cellular proteins during carcinogenesis; these may be demonstrated electrophoretically. The phenomenon is an expression of an essential difference between neoplastic and normal tissue and is not an indication merely of cellular activity.

3860

Louis, C.J. 1957. A study of carcinoma of the colon using a histochemical technique. Australian and New Zeal. J. Surg. 27:146-154.

A brief review of work in histological staining and recognition of malignant cells is given, followed by a resume of fluorescent antibody technique development. Sera preparation, absorption, and use are outlined. Results demonstrated some differentiation of neoplastic and non-neoplastic tissue in the intestine in a series of cases. Natural fluorescence of various tissues and secondary fluorescence are discussed. Distinction between cancerous and precancerous growth is pointed out. It was felt that the reaction observed was not a simple antigen-antibody phenomenon but rather a physicochemical one because of the special physicochemical characteristics of the protein involved. The significant meanings of this are outlined.

3870

Louis, C.J. 1957. The nature of leukemia: I. A histochemical study of the leukemic cell in man. Australasian Ann. Med. 6:300-310.

A component of the globulin fraction of rabbit serum reacts with the protoplasmic constituents of the normal cell but not with the corresponding malignant counterpart. The phenomenon can be demonstrated by fluorescent antibody; non-neoplastic tissues stain, but the neoplastic cells do not. This has been established particularly with experimentally produced hepatoma in rats and has been applied to naturally occurring tumors in man with similar results. Films obtained from normal individuals as well as from those suffering from infectious conditions were compared, using this technique, with those of leukemias. Normal white cells and those from various infectious diseases fluoresced well. Among the leukemias, the cells of chronic myeloid, chronic lymphatic, and monocytic leukemias all fluoresced brightly. A proportion of the cells of acute leukemias failed to fluoresce. This observation suggests that the chronic leukemias possibly are non-neoplastic conditions but that acute leukemias, from the point of view of this method of study, are neoplastic.

3880

Louis, C.J. 1958. The nature of leukemia: II. A histochemical study of the leukemic cell in the experimental animal. Australasian Ann. Med. 7:219-227.

The method of staining tissues with a fluorescein globulin complex, by which malignant tumor cells may be distinguished from normal cells, has been applied to leukemia of animals. It was also applied to tumors in animals, both induced by carcinogens and naturally occurring, as well as transplantable growths. Naturally occurring leukemia and that following irradiation were observed in the mouse. Distinction between the two types of leukemia was well defined, and as in man, the cells from chronic leukemia all stained well, but those from acute leukemia did not.

3890

Louis, C.J. 1958. Tumors of the breast, a study employing a histochemical technique. Brit. J. Surg. 46:147-155.

Tumors of the breast have been studied with a staining technique by which clear distinction can be made between normal and malignant cells. It has been shown, by using a globulin fluorescein complex stain, that normal tissue cells, mainly of epithelial type, stain well and can be demonstrated by fluorescence in ultraviolet light, but that cells of malignant tumors do not. Hyperplastic tissues and those of fibro-adenomata of the breast show the same features as normal tissue and thus are clearly distinguished from neoplastic tissue. This method has been applied to naturally occurring tumors in man and animals and the same distinction between neoplastic and non-neoplastic tissues has been observed uniformly. The method was first employed as an antigen-antibody reaction; globulin obtained from animals that had been injected with appropriate tissue was used as an integral part of the stain. Since a similar result can be achieved with a globulin fraction from any rabbit, this has been employed here.

3900

Louis, C.J. 1958. The significance of the cell type in the fluorescein globulin staining of tissues. Brit. J. Cancer 12:537-546.

Fluorescent serum protein may be used as a serologically nonspecific stain that stains normal but not malignant tissues. All normal tissues of vertebrates fluoresce brightly if there is a sufficient amount of protoplasm in the cells. This is due to the presence in the normal cell of a protein complex whose isoelectric point is sufficiently different from that of the serum proteins to allow interaction of the proteins. Normal cells that fail to stain are resting connective tissue cells that have little protoplasm, i.e., normal fibroblast; cells containing melanin pigment, obscuring protoplasm of the cell; red corpuscles where hemoglobin is most of the cell matter and the isoelectric point is too near that of proteins in the stain to allow combination; cells of the central nervous system, which constitute a special group. Malignant tissue fails to stain. The cells of such a tumor often contain voluminous protoplasm, but this does not show the phenomenon. Malignant cells may be distinguished from normal cells without real exceptions, excluding the normal red cell. The few exceptions are of a form and degree that will be resolved with improvements in technique.

3910

Louis, C.J. 1960. Fluorescein globulin staining of tissues. Med. J. Australia 2:707.

The fluorescent globulin stain was used as a histoserological stain to show that the cells of rat hepatoma had lost their organ-specific antigens. Normal liver cells reacted with the stain but cells of rat hepatoma did not. That the reaction was not an antibody-antigen phenomenon was shown by the observation that identical results could be obtained by using normal rabbit globulin, rabbit globulin, rabbit albumin, serum albumin fractions from other animals and even birds, and egg albumen. Since anion cation complexes were readily formed in mixtures of proteins, the formation of such salt-like complexes between stain globulin and cytoplasmic proteins could account for the staining reaction. This method had been employed in a wide variety of tumors and tissues in both man and animals

and, in particular, in a series of carcinomata of the colon, breast, skin, and uterus, in leukemias in man and animal, and in thyroid gland and cells in tissue culture. In all cases differential staining was observed between malignant cells and their normal counterparts. In some cases in which it was clear that malignancy was going to develop, areas of loss or diminution of staining were found.

3920

Louis, C.J. 1961. Fluorescein globulin staining of tumor transplants. Arch. Pathol. 72:593-598.

Fluorescent globulin complexes stain normal but not neoplastic tissues. Some staining of transplanted tumors occurs, although this is less obvious than that of normal tissues. Examination of a series of transplanted tumors has shown that the cytoplasm of these transplanted tumor cells fails to stain in the same way as do the cells of other neoplasms. The staining in these cells is a surface phenomenon due to absorbed globulin, which in turn reacts with the stain. The nature of this staining reaction is partly nonspecific and partly serologically specific, and thus differs from that seen in the differential staining between normal tissues and spontaneous or carcinogen-induced tumors.

3930

Louis, C.J.; Varasdi, G. 1960. Fluorescein protein globulin affinities of tumors of the thyroid gland. Ann. Surg. 152:795-803.

A histochemical method has been developed that will distinguish between malignant and nonmalignant tissues. Normal and hyperplastic tissues stain well, the stain fluorescing green under ultraviolet light; malignant tissues do not stain. In the thyroid gland malignant tumors do not stain by this method. Various types of nodules, or adenomata, showed well-defined staining. From what is known of the general characteristics of the nodules occurring in the ductless glands, it is improbable that there would be an absolute distinction between hyperplastic and neoplastic conditions; the one group might be expected to merge at some point into the other. The islands of loss of staining seen in preneoplastic states elsewhere were not found in this series. The value of the method as a routine procedure, once it has been simplified and thoroughly confirmed, is apparent.

3940

Nairn, R.C.; Richmond, H.G.; Fothergill, J.E. 1960. Differences in staining of normal and malignant cells by nonimmune fluorescent protein conjugates. Brit. Med. J. 2:1341-1343.

Investigation of the histological staining reaction of a variety of human and animal tumors with fluorescent conjugates of nonimmune rabbit globulin, NRG, has shown variable results, with a trend toward equal staining of normal tissue and benign tumors and diminished staining of carcinomas. Staining was never bright, and with the exception of human colon carcinoma, which stained less brightly than corresponding normal tissue, differential staining was not consistent. The technique cannot be regarded as a useful practical test for the diagnosis of malignancy. Centrifugation and electrophoresis experiments on homogenized samples of some tumors and corresponding normal tissues showed that the soluble rather than the particulate components are responsible for combining with

the conjugated NRG. Combination can take place with all the conjugate, which, possibly because of its net negative charge, behaves as a histological stain in much the same way as standard acid dyes. The diffuse nonspecific staining produced by conjugated NRG is different in kind and degree from the bright, defined staining obtained with conjugated organ-specific antisera.

3950

Nairn, R.C.; Richmond, H.G.; McEntegart, M.G.; Fothergill, J.E. 1960. Immunological differences between normal and malignant cells. *Brit. Med. J.* 2:1335-1340.

An alteration in cell constituents associated with malignant change has been investigated by immunological methods. Three types of normal and tumor tissue were compared: rat liver and hepatoma, hamster kidney and renal carcinoma, and human skin and various skin tumors. Antigen was prepared from homogenized normal tissue. Rabbit antisera were tested against the corresponding normal and tumor tissues by three methods, using appropriate tissue preparations to absorb and remove non-organ-specific antibodies: complement fixation, gel-diffusion analysis, and fluorescent staining by the sandwich technique. Control studies were carried out. There are organ-specific antigens in liver and kidney that are either absent or present in very small amounts in the corresponding tumor. In malignant tumors of skin a partial depletion of specific antigen was demonstrated; the fluorescent-antibody studies suggest that the specific antigen is localized in the cell membrane and in the perinuclear zone, with some faintly staining granules elsewhere in the cytoplasm. Lack of organ-specific antigen may indicate absence of a particular growth-controlling factor from malignant cells. The localization of the antigen to normal cell membrane may be related to surface identification of the cell.

3960

Pressman, D. 1961. Labeled antibodies in autochthonous tumor studies. *Cancro Torino* 14:425-432.

Antibodies were isolated that could localize in a chemically induced rat hepatoma. They localize to a high extent in the tumor and to a low extent in normal liver. Use of radio-labeled and fluorescent antibodies is described.

3970

Weiler, E. 1956. Antigenic differences between normal hamster kidney and stilbestrol-induced kidney carcinoma: Histological demonstration by means of fluorescing antibodies. *Brit. J. Cancer* 10:560-563.

The antigenic relationship between normal kidney and stilbestrol-induced kidney carcinoma has been studied, using fluorescein-coupled antiserum globulin against cytoplasmic particles and fresh frozen tissue sections. Fluorescent kidney-specific antibody does not give any staining reaction with tumor cells. This is in accordance with preceding findings, in which no kidney-specific antigen could be found in tumor tissue. In normal kidney tissue, the kidney-specific antibody reacts with the cytoplasm of the tubular epithelium only. In the proximal convoluted tubules, there is a concentration of kidney-specific antigen in the brush border. Unabsorbed fluorescent kidney antiserum gives, in addition to the staining of normal tubular cells, a relatively weak staining with tumor cells. Fluorescein-coupled antiserum against tumor particles strongly stains both normal epithelial cells and tumor cells, and gives a weak reaction with glomeruli.

B. GENERAL METHODS

3980

Anonymous. 1958. Antibody-antigen reactions observed by fluorescence. *Lancet* 2:111.

A brief review is presented of the use of the fluorescent antibody technique in the diagnosis of infectious diseases. Globulin affinities and their use in tumor detection are mentioned. Some of the problems encountered with the technique, especially in examining a very heterologous material such as feces, are discussed, and possible future uses of the method are pointed out.

3990

Fitch, F.W. 1962. Immunohistochemical study of Ehrlich ascites tumor. *Arch. Pathol.* 73:144-160.

Antisera were prepared in rabbits against Ehrlich ascites tumor cells and whole mouse blood, and their globulins were labeled. These conjugates were used to study staining of Ehrlich ascites tumor cells, mouse blood, liver, renal, and other tissues. Absorption studies were made. Only the cell surface of intact tumor cells stained. Observations indicated that antibodies were responsible for cytotoxic effects on the tumor cel

4000

Friend, C.; Rapp, F. 1962. Intracellular localization of Swiss mouse leukemia virus. *Federation Proc.* 21:454.

Previous studies reported the development of immunologic methods useful in characterizing the virus that causes leukemia in adult Swiss mice. In this study, immune sera were obtained from mice that survived challenge with live virus after vaccination with formalinized virus. The immune sera were evaluated by neutralization and immunofluorescent methods and, in some instances, by hemagglutination inhibition. All tests correlated well: the latter two yielded information rapidly that served as an index for the usefulness of each serum. Titers were generally low but specificity was established in relation to normal sera, normal tissues, and material from unrelated mouse neoplasms. The immune sera were used with rabbit antimouse globulin to study localization and spread of the virus in mouse cells and tissues by immunofluorescence. Virus components were detected in the nucleus and cytoplasm of infected cells; virus antigen appeared first in the nucleus and then in the cytoplasm, as with the myxoviruses. Thus, immune sera against the Swiss mouse leukemia virus were useful in studies of the formation of virus components and infectious virus.

4010

Girardi, A.J.; Slotnick, V.B.; Hilleman, M.R. 1962. Search for virus in human malignancies: I. In vitro studies. *Proc. Soc. Exp. Biol. Med.* 110:776-785.

Fluorescent antibody employing rabbit antihuman globulin was one of the observation methods used in the subject study. Results by all methods, including FA, were negative.

140

4020

Girardi, A.J.; Sweet, B.H.; Slotnick, V.B.; Hilleman, M.R. 1962. Development of tumors in hamsters inoculated in the neonatal period with vacuolating virus, SV40. Proc. Soc. Exp. Biol. Med. 109:649-660.

Intracerebral and subcutaneous injection into newborn hamsters of vacuolating virus, SV40, grown in renal cell cultures of grivet monkeys resulted in single or multiple fibrosarcomas at the site of injection that were histologically of varying degree of malignancy. These occurred 3.5 to 8 months postinoculation and in both sexes. Animals injected with appropriate control materials or held uninoculated failed to develop tumors. Tests to exclude mouse polyoma virus as a factor were clearly negative. Evidence for the role of SV40 virus as a primary oncogenic agent was provided by recovery of the virus from the tumor and by demonstration of SV40 antigen in tumors by fluorescent antibody staining. The agent appeared to be localized in the tumors because the virus was not detected in blood or excretions, antibody response was minimal or lacking, and gross metastases were lacking. Transplantation and serial passage of SV40-induced tumors were accomplished with ease. The data represent the first definitive evidence implicating a virus of primate origin as a malignant oncogenic agent in experimental animals.

4030

Gluck, E. 1962. Fluorescent antibodies in cancer research: A review. Cancer Res. 22: 895-897.

Review.

4040

Goldstein, M.N.; Hiramoto, R.; Pressman, D. 1959. Comparative fluorescein labeling and cytotoxicity studies with human cell strains HeLa, Raos, and 407-liver and with fresh surgical specimens of cervical carcinoma, osteogenic sarcoma, and normal adult liver. J. Nat. Cancer Inst. 22:697-706.

Antisera prepared against a human melanoma and against normal human thyroid gave the same staining and cytotoxic reactions as antisera to two permanent human cell lines, HeLa and 407-liver. Rabbit antiserum reacted primarily with connective tissues in non-cultured surgical specimens. Antimelanoma serum showed a staining reaction with both HeLa and Raos cultures and with the tumor cells of cervical carcinoma and osteogenic sarcoma tissues obtained from specimens for biopsy. This indicated that similar antigens are present in the tissue culture cells and in cells of the same type of tumor from which they were derived. Hepatic cells did not stain with the antiserum, although good staining could be demonstrated in cultures of 407-liver strain. This suggested that the liver strain may have originated from connective tissue rather than from embryonic parenchyma in the liver.

4050

Herbeuval, R.; Herbeuval, H.; Duheille, J. 1962. Fluorescence and immunofluorescence in blood cytology. Strasbourg Med. 13:621-626. In French.

Applications of fluorescence microscopy and FA in the study of white cell concentrations for evidence of cancer are discussed. A review of the results of other workers and those of the authors is presented.

4060

Hillemanns, V.H.G. 1962. Serological and immunohistological investigations on the development of cancer. Z. Naturforsch. 17b:4:240-261. In German.

Biochemical differences between normal pavement epithelium and cancerous tissue are investigated by serological and immunohistological methods. Emphasis is on the comparison of human cancer cells with their mother cells of the same organ by consideration of the phenomenon of organ specificity.

4070

Hughes, P.E. 1958. The significance of staining reactions of preneoplastic rat liver with fluorescein globulin complexes. Cancer Res. 18:426-432.

During aminoazo dye carcinogenesis, islands of morphologically normal parenchymal cells could be found in the livers of most rats that had lost their affinity for fluorescein rabbit globulin complexes. This stain characteristic persisted long after the carcinogen was discontinued. In any group of rats on a particular aminoazo carcinogen, there is a wide variation in the rate at which these areas appear. However, in an investigation with a variety of dyes, a correlation was found between the rate of appearance of these islands and the carcinogenicity of the particular dyes fed. This staining reaction depended upon protein-protein interactions. The more basic of the soluble cytoplasmic proteins, which include most of the carcinogen-binding proteins investigated by Miller and Miller, could be implicated. The observations lend indirect support to the protein-deletion hypothesis. Since differential staining of neoplastic and non-neoplastic tissues could be observed with fluorescein-conjugated normal rabbit globulin, any interpretation of the results obtained by fluorescent antibody technique will require great caution and very careful control staining before changes can be attributed to an antibody-antigen reaction.

4080

Hughes, P.E.; Louis, C.J. 1957. Role of organ-specific antigen during 4-dimethylaminoazobenzene carcinogenesis in rat liver. Nature 180:289-290.

Studies are reported of the role of the organ-specific antigen during 4-dimethylaminoazobenzene carcinogenesis in the rat liver, using the fluorescent antibody test and complement fixation.

4090

Hiramoto, R.; Bernecky, J.; Jurandowski, J.; Pressman, D. 1961. Immunohistochemical staining properties of the N-2-FAA rat hepatoma. *Cancer Res.* 21:1372-1376.

Hepatomas induced in rats by feeding N-2-fluorenylacetamide, N-2-FAA, are not stained by fluorescein-labeled antibody to rat liver microsomes. This indicates that components of normal liver microsomes were not observed in the hepatoma. Some transplantable tumors derived from liver tissue, the Miller hepatoma, Novikoff hepatoma, and the Simpson reticulum-cell sarcoma also do not react with the fluorescent antibody system. The use of nonimmune fluorescent conjugate as described by Hughes et al. to differentiate tumor elements from normal components by nonspecific staining technique did not show consistent lack of staining of tumor tissues in our hands.

4100

Hiramoto, R.; Jurand, J.; Bernecky, J.; Pressman, D. 1962. Lack of staining of testicular tumors by antisperm and antitestis antibodies. *Proc. Soc. Exp. Biol. Med.* 111: 505-507.

The fluorescent antibody technique was employed to determine the presence of normal antigens of sperm, seminal fluid, and testis in various testicular tumors. Ten testicular tumors showed no staining with any of the antisera to the above normal antigens. These results show that there are components unique in testis that can be stained with antibody against testis, seminal fluid, and sperm but these components were not present in the several testicular tumors tested at a concentration high enough to be observed. Some differences were noted between the antiseminal fluid, antisperm, and antitestis sera. The three sera reacted with sperm cells. Antisperm and antitestis, in addition to staining sperm cells, reacted with stromal elements in testis. The latter serum also contained antibodies to germinal epithelium.

4110

Hiramoto, R.; Nungester, W.J. 1958. Penetration of serum globulins into mouse tumors. *Cancer Res.* 18:27-32.

The intravenous injection of normal or immune globulin tagged with iodine 131 into mice bearing the Ehrlich tumor resulted in some increase in radioactivity in the tumor over that found in the spleen, liver, or kidney. The fluorescein-tagged normal and immune sera were not localized to any significant degree in the tumor. However, tumor cells could be made to fluoresce in vivo with tagged immune globulin, provided that the cells were first injected intravenously and were trapped in the blood capillaries of the lung.

4120

Hiramoto, R.; Yagi, Y.; Pressman, D. 1959. Immunohistochemical studies of antibodies in anti-Murphy lymphosarcoma sera. *Cancer Res.* 19:874-879.

That anti-ascites Murphy lymphosarcoma antibodies, anti-ALS, and antirat fibrin antibodies localize in the solid Murphy lymphosarcoma of rats when injected intravenously has previously been shown by the use of radioiodine-labeled antibody. It was found by

immunohistochemical technique that the localization patterns of these two antisera *in vivo* after intravenous injection were essentially identical. Localization took place in the connective tissue stroma of the tumor mass but not in or on the cells themselves. Three other transplanted rat tumors showed the same localization. *In vitro*, both sera stained the stroma, but the anti-ALS serum stained the cell contents of the lymphosarcoma cells as well. Cells of other tumors were not stained. The periphery of the intact ascites tumor cell was stained uniformly by anti-ALS antibodies. Staining by antifibrin, on the other hand, was sporadic and quite similar to control staining. Normal tissue sections treated with antifibrin showed some extracellular staining. Anti-ALS serum, in addition, stained lymphoid cells in spleen, thymus, and lymph node, indicating the presence of cross-reacting components.

4130

Ito, M. 1960. Studies on viral oncolysis by means of fluorescent antibody technique. Jap. J. Bacteriol. 15:601-610. In Japanese.

Fifteen different types of dye compounds with various kinds of conjugating radicals were evaluated for adequacy for the fluorescent antibody technique. Three practical fluorescent antibodies were prepared, using DNS, XRB, and a sample fluorescent dye from cyanulyl chloride. DNS fluorescent antibody has a yellow fluorescence, XRB a tannish-red, and cyanulyl chloride dye a yellowish-green bright fluorescence. Each of them can easily conjugate with protein and causes no denaturation of protein during the reaction. The characteristics of these labeled proteins were investigated with a Beckman spectrophotometer and spectrofluorescent-photometer. Labeling the dyes and the latest technique of the fluorescent antibody preparation were discussed.

4140

Ito, M.; Nishioka, K. 1959. Two reagents for fluorescent antibody technique and visualization of the viral antigen synthesized in Ehrlich ascites tumor cells infected with ED virus: Studies on viral oncolysis. Jap. J. Microbiol. 3:71-83.

A fluorescent antibody technique using 1-dimethylaminonaphthalene-5-sulphonylchloride and xylene Red B has been described. The conjugation procedure is simple, without deterioration of antibody activity. The quality of each conjugate was determined with a Beckman spectrophotometer and spectrofluorometer. Viral antigen synthesized in Ehrlich ascites tumor cells infected with ED virus was distinctly visualized by staining of each conjugate, and specificity was confirmed. XRB-conjugated protein was preferable because it showed brilliant orange fluorescence and could be clearly discriminated from the auto-fluorescence of cells. Viral antigen was detected in the cytoplasm of infected cells within 6 hours after virus challenge. Maximum was reached at 24 hours. FA observation confirms previous results obtained by immunochemical and cell fractionation studies.

4150

Korngold, L.; Lipari, R. 1955. Tissue antigens of human tumors grown in rats, hamsters, and eggs. Cancer Res. 15:159-161.

A human epidermoid carcinoma, Hep 3, and a human sarcoma HS 1, that had been grown for many generations in rats and eggs as well as in hamsters, Hep 3 only, were analyzed by

the method of Ouchterlony for the presence of human antigens. The same human antigens were always detected, regardless of the number of generations that the tumor had been grown in the foreign hosts studied. Attempts to demonstrate that the human tumor might synthesize the tissue protein of the rat foreign host were negative.

4160

Levy, H.B.; Brodsky, I. 1959. Some biochemical effects of infection with Friend leukemia virus. Ann. N.Y. Acad. Sci. 81:51-61.

As a portion of this study, the direct fluorescent antibody technique was used to locate leukemia virus in infected spleens. Cytoplasmic staining predominated, with some nuclear and cell membrane involvement.

4170

Mellors, R.C. 1958. Viruses, genes, and cancer. Federation Proc. 17:714-723.

This paper discusses the basic concepts of cancer etiology, specifically the virus causation concept or extrinsic factor versus the somatic or gene mutation concept or intrinsic factor. The neoplasms primarily discussed are Rous sarcoma, fowl leukemias, and particularly Shope papilloma of rabbits. Antibodies were obtained against the Shope papilloma virus, tagged with fluorescein, and histological studies were made with the tagged antibody and hematoxylin-eosin stains. Development of the virus was followed. An incomplete versus complete virus antigen is postulated because the virus was not demonstrated in the proliferating cells of the cancer but was readily evident in older areas. It was postulated that an incomplete virus, although actually an extrinsic factor in cancer etiology, would be indistinguishable by fluorescent antibody and certain other techniques from a true intrinsic factor.

4180

Mellors, R.C. 1960. Virus etiology of cancer. Bull. N.Y. Acad. Med. 36:415-418.

A general discussion of virus etiology of Shope papillomas and related cancers is presented. Elucidation of features of the disease by FA methods is the prime study method.

4190

Midgley, A.R.; Fierce, G.B. 1962. Immunohistochemical localization of human chorionic gonadotropin in embryonal carcinoma and trophoblastic tissues. Federation Proc. 21:198.

With the use of fluorescein-labeled antibody, human chorionic gonadotropin, HCG, has been localized to syncytiotrophoblastic cells of immature placenta, hydatidiform mole, chorioadenoma destruens, and choriocarcinoma. No HCG has been detected in cytotrophoblasts. Since formalin-fixation did not alter the ability of HCG to react with its antibody, these observations were made on formalin-fixed paraffin-embedded tissues and confirmed with fresh frozen tissues. In previous studies, attempts to direct the differentiation of a human embryonal carcinoma toward choriocarcinoma with colchicine or X-irradiation resulted in the appearance of giant cells that superficially resembled syncytiotrophoblastic cells of choriocarcinoma.

4200

Noyes, W.F. 1959. Studies on the Shope rabbit papilloma virus: II. The location of infective virus in papillomas of the cottontail rabbit. *J. Exp. Med.* 109:423-428.

A method has been devised to determine the location of infective Shope virus in the papillomas of cottontail rabbits. Frozen sections of the growths were burned selectively with a microcautery to destroy either the keratinized or proliferating layer, and the sections were then applied directly to the sensitized epidermis of domestic rabbits. No papillomas appeared when the keratohyaline and keratinized areas had been eliminated leaving the proliferating cell layer, but papillomas arose when proliferating cell areas were destroyed leaving the keratohyaline and keratinized layers. The results indicate that infective Shope papilloma virus is situated mainly, perhaps entirely, in the keratohyaline and keratinized areas of cottontail papillomas. This is in accord with the previous disclosure by the fluorescence technique that virus antigen in demonstrable quantity is present only in these sites.

4210

Rapp, F.; Friend, C. 1962. Detection of cytoplasmic deoxyribonucleic acid and nucleoproteins with antinuclear serum. *Virology* 17:497-499.

Antinuclear sera and the immunofluorescent method for the detection of virus nucleoproteins and DNA in the cytoplasm of ascites cells, smears of solid tumors, and cultured cells are described.

4220

Rapp, F.; Steinglass, M.; Friend, C. 1962. Hemagglutination by Swiss mouse leukemia virus. *Bacteriol. Proc.* V21:134.

Detection of the virus causing leukemia in adult Swiss mice has been based on infectivity titrations and on survey of tissues by electron microscopy. Conditions for the demonstration of leukemia virus hemagglutination have now been determined. Filtrates from leukemic spleens and subcutaneous tumors harvested at various times following inoculation were the source of virus. Hemagglutination occurred at 37°C, at room temperature, and at 4°C when the reaction was carried out in Sorensen buffer adjusted to a pH of 5.5 to 5.6. This activity was lost at higher pH. Erythrocytes from various species were equally effective. Heating at 45°C for 30 minutes appeared to enhance the effect. Titers were generally low but consonant with both infectivity and immunofluorescent determinations for presence of virus in the tissues. Extracts and filtrates from normal mouse tissue served as controls. Agglutination was inhibited by antiviral mouse serum but normal mouse serum failed to cause marked reduction in hemagglutinating activity. Development of a simple test for detection of virus hemagglutinin has aided the study of the pathogenesis of a virus-induced murine leukemia.

146

4230

Rifkind, R.A.; Osserman, E.F.; Hsu, K.C.; Morgan, C. 1962. The intracellular distribution of gamma globulin in a mouse plasma cell tumor X5563 as revealed by fluorescence and electron microscopy. *J. Exp. Med.* 116:423-432.

Ferritin- and fluorescein-conjugated antibody staining has been applied to a study of a mouse plasma cell tumor. The presence of myeloma globulin within cisternae of the endoplasmic reticulum was observed at a stage of the secretory process when the remainder of the cytoplasm was essentially free of labeled globulin. The distribution of ferritin suggested a functional heterogeneity among units of the endoplasmic reticulum. Apparently, progressive accumulation of globulin results in distension of the endoplasmic reticulum and, occasionally, in the appearance of considerable quantities of this secretory protein in the extracisternal cytoplasmic matrix. Participation of the Golgi apparatus in the packaging and release of small quantities of globulin seems likely. In addition, however, fragmentation of the peripheral cytoplasm, with rupture of distended ergastoplasmic vesicles, appeared to be another pathway whereby globulin is secreted.

4240

Rubin, H. 1962. Response of cell and organism to infection with avian tumor viruses. *Bacteriol. Rev.* 26:1-13.

In a portion of this study of Rous sarcoma virus infection and resistance-inducing factor, fluorescent antibody was used to follow the infection at the cellular level. Viral antigen was first detected at 2 days along the border of cells. FA demonstrated the infection to be at the surface, not in the cells. Viral antigen was subsequently shed from the cells into a surrounding matrix. Still later, the viral antigen appeared within the cytoplasm.

4250

Rubin, H.; Vogt, P.K. 1962. An avian leukosis virus associated with stocks of Rous sarcoma virus. *Virology* 17:184-194.

Isolation of a second virus from stocks of Bryan high-titered strain of Rous sarcoma virus, RSV, is reported. The new agent, Rous-associated virus, RAV, can be detected in tissue culture, despite failure to produce discrete cytological alterations, because it interferes with infection and focus formation by REV. There is a strong selection for RAV when cells infected with RSV are cloned, so that most of the clones produce much RAV and little RSV. In stocks of Bryan strain, the titer of RAV is about four times that of RSV. RAV induces resistance to RSV much more rapidly than does resistance-inducing factor, a naturally occurring leukosis virus. RAV can be assayed in vitro. RAV is more closely related immunologically to RSV than to resistance-inducing factor. FA studies show that it matures at the cell membrane, as does RSV. In the electron microscope RAV is morphologically indistinguishable from RSV and other chick tumor viruses. It produces no tumor at the site of inoculation in chickens, but produces erythroblastosis following intravenous inoculation of embryos.

4260

Seelich, F.; Kaiser, E.; Springer, K. 1962. Cytotoxic effect of antiserum against a dye-protein complex. Experiments with Ehrlich ascites tumor of the mouse. Z. Krebsforsch. 64:6:448-458. In German.

The fluorescent dye sulforhodamine B was bound to human and to bovine albumin; this dye-protein complex was then injected into rabbits. In this way an antiserum was produced that reacted with vitally stained ascites cells of the Ehrlich ascites tumor of the mouse, i.e., vital staining after injection of the dye. In the presence of active complement the serum had a cytotoxic action. After vital staining of the solid Ehrlich tumor, an injection into the tumor of the antiserum against the dye component caused characteristic necroses. These could be distinguished readily from spontaneous necroses occurring in the tumor in the control animals. This necrotizing effect was associated with an intact complement system. After similar treatment with normal rabbit serum no such cell or tissue changes could be observed.

4270

Soules, D.E.; Surereker, A.; Vitek, G.; Fugate, K. 1962. Presence of structures resembling viral inclusion bodies in spontaneous AKR mouse leukemia. Bacteriol. Proc. V22: 134.

A subcellular fraction of homogenized thymus tissue from a leukemic AKR mouse was injected into rabbits. Antiserum, after conjugation with fluorescein isothiocyanate, was used for examination of thin frozen sections of thymus and other tissues from leukemic and nonleukemic AKR mice. Small brightly fluorescent bodies were noted both intra- and extra-cellularly in thymus but in no other tissue. Acridine orange, AO, revealed these bodies to be DNA. DNAase removed the AO staining of cell nuclei but not of the bodies. Pepsin treatment followed by DNAase was necessary to remove AO staining, giving evidence of similarity to viral inclusion bodies. Extracellular structures were adjacent to disintegrating thymus cells. An occasional body could be found in preleukemic animals but were frequently found in animals with clinical disease. Two-month-old mice in which leukemia was produced early by intraperitoneal injection of thymus cells from leukemic mice had most of the inclusion bodies. They were not in nonleukemic Swiss or C57 mice. Cell-free extracts of thymus tissue from leukemic animals injected into preleukemic AKR, nonleukemic Swiss, and C57 mice gave no evidence of leukemia or tumors of any type.

4280

Southam, C.M.; Noyes, W.F.; Mellors, R.C. 1958. Virus in human cancer cells in vivo. Virology 5:395-398.

During clinical trials of virus infections as experimental cancer therapy the fluorescein-labeled antibody technique has been utilized as an adjunct to virus isolation, i.e., inoculation of 30-day-old mice, for the study of virus oncotropic. These studies emphasize the difficulty of establishing an etiologic diagnosis by virus isolation, since living intracellular virus may not be transmissible to a new host. Conversely, they demonstrate the impossibility of establishing an etiologic significance for microbial inhabitants of disease tissue until all of the Koch postulates are fulfilled.

4290

Takahashi, M.; Kato, S.; Kameyama, S.; Kamahora, J. 1959. A study on the multiplication of rabbit myxoma virus with the fluorescent antibody technique. P'kens J. 2:333-340.

FL cell cultures infected with rabbit myxoma virus were studied by the fluorescent antibody technique and by light microscopy. The findings were correlated with the growth curve of the infectious virus, and development of complement-fixing antigen was determined from companion cultures. By restaining the fluorescent cells with Giemsa solution, it was shown that B-type inclusion bodies of this virus consisted of viral antigens. Furthermore, the appearance of fluorescent cells and inclusion bodies just before the increase in infectivity suggested that these inclusion bodies might be the main site of virus synthesis in the cells. No distinct fluorescence was seen in the nucleus at any stage of infection, implying that the virus synthesis took place exclusively in the cytoplasm.

4300

Vazquez, J.J. 1956. Immunocytochemical study of plasma cells in multiple myeloma. J. Lab. Clin. Med. 51:271-275.

FA was used to show that plasma cells from patients with multiple myeloma contain cytoplasmic concentrations of gamma globulin. Similarly, non-neoplastic plasma cells from hyperimmune animals showed concentrations of gamma globulin and specific antibody.

4310

Weiler, E. 1956. Alteration of liver-specific serology in the rat during carcinogenesis by means of p-dimethylaminoazobenzol. Z. Naturforsch. 11b:1:31-38. In German.

The author has confirmed histoserologically, with the aid of liver-specific fluorescein-labeled antibodies, that the liver-specific antigen cannot be demonstrated in primary rat liver cell carcinoma. The liver-specific antigen is localized exclusively in the parenchymal cells of the liver. Liver tissue exposed to the carcinogen contains less liver-specific antigen than the normal liver. This is expressed in the complement-fixation test by a reduced liver-specific serologic activity of the microsomes and mitochondria, and histoserologically in the appearance of characteristic tissue islands in which the antigenic concentration is decreased in comparison with the neighboring tissue. The degree of antigenic loss depends upon the total dose of carcinogen administered and on the latent period.

4320

Yohn, D.S.; Hammon, W.M.; Atchison, R.W.; Casto, B.C. 1962. Serial heterotransplantation of human adenocarcinoma No. 1 in the cheek pouch of unconditioned adult Syrian hamsters. Cancer Res. 22:443-448.

This report describes studies establishing that the Yoolan human adenocarcinoma No. 1, H.Ad., a human tumor transplantable in conditioned laboratory animals, could be serially transplanted in cheek pouches of normal unconditioned hamsters and, under these conditions,

for more than 60 serial passages has retained proliferating human tumor tissue. Tumor passaged in normal hamster is called H.Ad. 1U. Histologically, H.Ad. 1U closely resembles early generations of H.Ad. 1 in conditioned animals. Human hemagglutinins were produced by guinea pigs immunized with either H.Ad. 1U tumor or cell culture line; human cellular antigen was demonstrated in H.Ad. 1U tumor cells and cell culture lines by two independent indirect fluorescent antibody systems. The karyologic state of H.Ad. 1U tumor apparently ranges from subdiploid human to polyploid human. Some potential factors that may account for the apparent indefinite passage-ability of this tumor in unconditioned hamsters are presented.

AUTHOR INDEXA

Abbott, J.	2870	Becker, B.	2130*, 2140*
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		Buchanan, W.W.	690

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Kurtz, S.M.	3210*, 3230	McElhinney, A.J.	890
Kushner, I.	470	McEntegart, M.G.	2960, 3240, 3950
Kyogoku, M.	2330, 2580*	McGarry, E.E.	3510, 3520
		McIvor, B.C.	3600
<u>L</u>			
Lacy, P.E.	3470*, 3480*, 3490*	McKay, D.G.	1990*, 2590*
Lack, R.G.	2280	McKenna, J.L.	550*
Lambotte, R.	3630	McKinnon, G.E.	2600*
Landing, B.H.	950, 1880	McPherson, S.E.	1100
Lange, K.	1100*, 1110*	Medzon, J.	2980
Laxson, C.	2520	Meiselas, L.E.	520, 560*
Leduc, E.H.	1820, 1940*, 2320, 2410		
Lee, L.	1950*		
Lee, R.E.	1460*		
Lee, S.L.	520*, 560, 870		

Mellors, R.C.	490, 570*, 580*, 590*, 600*, 610*, 620*, 1150*, 1160*, 1170*, 1180*, 1190*, 1200*, 1220, 2010, 2020, 2480*, 2930*, 4170*, 4180*, 4280	<u>P</u>	
Meneghelli, V.	3580*	Pachas, W.N.	40
Metzger, H.	1310	Pakesch, F.	130
Meyserian, M.	450, 460, 470	Parker, R.L.	640*
Midgley, A.R.	3590*, 4190*	Paronetto, F.	650*, 2270*, 3250*
Moeller, G.	2940*	Patterson, R.	2490*
Moller, H.F.	370	Payne, L.C.	2030*
Mongar, J.L.	2720	Pearse, A.G.E.	2360
Moon, H.D.	3600*	Pepe, F.	2920
Moran, T.J.	3230*	Perlmann, P.	3100
Morgan, C.	3080, 4230	Peters, J.	1050
Movat, H.Z.	630*	Peterson, W.C., Jr.	660*
<u>N</u>		Pettit, M.D.	960
Nace, G.W.	2950*, 3610*	Pierce, G.B.	3590, 4190
Nagai, H.	2370*	Pisciotta, A.V.	550
Nagy, C.F.	120	Pollack, A.D.	2570
Nairn, R.C.	1210*, 2960*, 3240*, 3620*, 3780, 3940*, 3950*	Pollack, V.E.	530, 670*
Nakano, H.	2370	Popper, H.	2150, 2270, 3250
Nastuk, W.L.	750	Porteous, I.B.	1230*
Neil, A.L.	2000*, 2380*, 2510	Potter, J.L.	1230*
Nemirovsky, M.S.	2910, 3220	Pressman, D.	20
Nishioka, K.	4140		1170, 1180, 2390*, 2820, 2830, 2840,
Noble, J.	1110		2850, 2930, 3010,
Nowoslawski, A.	580, 590	Pruzansky, J.J.	3790, 3800, 3810,
Noyes, W.F.	620, 4200*, 4280	Pulvertaft, R.J.V.	3960*, 4040, 4090,
Nungester, W.J.	4110		4100, 4120
<u>O</u>			
O'Brien, T.F.	1410	Rapola, J.	2650
Ohta, G.	2150, 2250*, 2260*	Rapp, F.	680*, 690*, 4000,
Cort, J.	1580, 2470, 2610*, 2660	Rappaport, B.Z.	4210*, 4220*
Ortega, L.G.	600, 610, 620, 1200, 1220*, 2010*, 2020*	Raskin, J.	2040*, 2620*, 2630*
Osserman, E.F.	4230	Reddy, C.R.R.M.	2640*
Osserman, K.E.	750	Reed, T.E.	3150
Ouchi, E.	3670	Rezaian, J.	1480*
		Richman, S.M.	530
		Richmond, H.G.	520, 560
		Richter, M.	3940, 3950
		Ricken, D.	2980
		Rifkind, R.A.	940
		Rigler, R.	3030, 4230*
		Rivero, H.H.	130
		Roberts, A.N.	880
			2050*

Roberts, D.St.C.	2970*	Shepherd, W.E.	760, 3320		
Robertson, C.E.	3320	Shirley, W.	3660, 3670		
Robinson, A.R.	1530	Shorter, R.G.	2230*		
Rodenburg, J.M.	3030, 3040	Shurin, S.F.	740*		
Rodnan, G.P.	240, 1030	Siegel, M.	560, 870, 1170,		
Roitt, I.M.	190, 360, 900, 1020, 1060, 1070, 1240, 1250*, 1260*	Silber, R.	1180, 2930 1680*		
Rose, B.	2980, 3510, 3520	Singer, E.J.	2150, 2250, 2260		
Rose, N.R.	950	Simons, B.E.	3350		
Rosen, F.S.	2180	Slotnick, V.B.	4010, 4020		
Rosenfield, R.	2250	Smolian, V.	3630		
Rothbard, S.	703*, 710*, 720*	Sokal, G.	1690*		
Rothenberg, M.S.	1280, 1310	Sonkin, L.S.	1190		
Rother, K.	2990	Sotelo, S.J.	1470		
Rothman, W.	2230	Soules, D.E.	4270*		
Rozansky, R.	2210	Southam, A.L.	1485		
Rubin, E.	2270	Southam, C.M.	4280*		
Rubin, H.	4240*, 4250*	Sparks, R.D.	3190		
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<u>S</u>					
Sachs, M.V.	90	Springer, K.	4260		
Salmon, J.	3630*	Steblay, R.W.	3290*, 3300*		
Salzman, R.T.	3190	Steiner, J.W.	3310*		
Santamaria, A.	3160	Steiner, R.F.	3640*		
Sargent, A.U.	2980*	Steinglass, M.	4220		
Sarre, H.	2990*	Stockdale, F.E.	3000*		
Saxen, L.	2420, 2650	Stoloff, I.L.	1290*		
Sbarra, A.J.	40, 3660*, 3670*	Strauss, A.J.L.	750*		
Scapinelli, R.	3580	Strauss, L.	650, 2250		
Scevola, M.E.	3400	Stuart-Smith, D.A.	3130		
Schaffner, F.	3250	Surereker, A.	4270		
Schild, H.O.	2720	Suszko, I.M.	2490		
Schilling, R.F.	140, 3700	Suyama, T.	3610		
Schlippkoter, H.W.	2500*	Sweet, B.H.	4020		
Schmid, F.R.	640	Szulman, A.E.	1490*, 1500*, 1510*		
Schuit, H.R.E.	370	<hr/> <u>T</u>			
Schwab, F.	1780	Takahashi, M.	4290*		
Scott, D.G.	3260*, 3270*	Takahashi, S.	2330		
Seegal, B.C.	730*, 750, 1270*, 1280*, 1310, 3080,	Tamanai, I.	3010*		
Seelich, F.	3280*	Tanaka, N.	2410*		
Sehon, A.H.	4260*	Taylor, H.E.	760*, 3320*		
Seip, W.F.	3410	Teague, P.	180		
Sengson, B.L.	1770	Tenczar, F.J.	1520		
Senterfit, L.B.	590	Teodoru, C.V.	1300*		
Seybold, G.	2570	Thayer, W.R.	170		
Shahani, S.	2100	Thompson, G.R.	770*		
Shames, J.M.	1485*	Tjio, J.H.	500		

Tobie, J.E.	500	Went, H.A.	3680*
Toivonen, S.	2420, 2650	West, C.W.	960
Toy, B.L.	50, 60, 1750	Whipple, A.	1880
Triedman, R.S.	1310*	Whitaker, J.	1530*
Tunik, B.	3020*	White, R.G.	840*, 850*, 860*, 1320*, 1330*, 1340,
Turner, W.A.	2770		1720, 2070*, 2080*, 2090*, 2350, 2440*
Turrubiarte, V.	40		
<u>U</u>			
Unger, R.H.	3350	Widelock, D.	870*
Urquhart, J.A.	1210, 1310	Williams, E.	850
<u>V</u>			
Vainio, T.	2420*, 2650*	Winnick, T.	3050*
van den Berg, C.	2660*	Wise, L.J.	2310*
van Rijssel, Th. G.	2610, 2660	Wissler, R.W.	3330*
Varasdi, G.	3930	Witebsky, E.	920, 930, 940, 950
Vauhan, J.H.	480	Witmer, R.H.	2670*
Vazquez, J.J.	240, 780*, 790*, 800*, 970, 990, 1000, 1030, 1460, 1710*, 1730, 1840, 2060*, 2290*, 2510*, 3150, 3230, 3690, 4300*	Witmer, V.R.	2680*
Vilar, C.	1140, 1960, 1970, 1980, 2910, 3220, 3540	Wollensak, V.J.	2100*
Vitek, G.	4270	Wood, C.	1340*
Vogt, P.K.	4250	Wood, R.M.	210, 1120
Volk, B.W.	1300	Woolf, N.	2110*, 2120*, 2170, 2240
<u>Y</u>			
		Yagi, Y.	2390, 2840, 2950, 3010, 4120
		Yakulis, V.J.	1910
		Yamada, A.	1090, 1450
		Yamasawa, K.	1540*
		Yavorovskaya, B.Ye.	740
		Yeager, J.A.	3060*
		Yohn, D.S.	4320*
		Young, M.R.	2730
<u>Z</u>			
Waaler, E.	810*	Zak, F.G.	2260
Wachstein, M.	1100, 1110	Zampa, G.A.	3360, 3370, 3530
Waksman, B.H.	2430*	Ziff, M.	100
Walzer, R.A.	2300*	Zingale, S.B.	520, 560, 880*
Wartman, W.B.	1520*	Zlotnick, A.	3690*
Watson, R.F.	700, 710, 720	Zuelzer, W.W.	1360, 1370, 1530
Weigle, W.O.	1000, 1560, 1570, 1840, 2460, 2540, 2550		
Weil, A.J.	3030*, 3040*		
Weiler, E.	3970*, 4310*		
Weir, D.M.	390, 400, 820*, 830*		
Weiser, R.S.	2520*		
Wenk, E.J.	1110		

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